

THE UNIVERSITY OF LIVERPOOL

ANNALS
OF
TROPICAL MEDICINE AND
PARASITOLOGY

ISSUED BY THE
LIVERPOOL SCHOOL OF TROPICAL MEDICINE

Edited by

PROFESSOR WARRINGTON YORKE, M.D., F.R.C.P., F.R.S.

PROFESSOR D. B. BLACKLOCK, M.D., D.P.H.

PROFESSOR W. S. PATTON, M.B.

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VOLUME 31

(April 8th, 1937, to December 21st, 1937)

With Frontispiece, nine plates, one map and eighty-seven figures in text

LIVERPOOL :
THE UNIVERSITY PRESS OF LIVERPOOL.

H. R. GRUBB, LTD.,
PRINTERS,
POPLAR WALK, CROYDON.

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Volume 31

April 8th, 1937

No.1

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- (1) Two courses for the Diploma in Tropical Medicine, commencing on the 30th September, 1937, and the 3rd January, 1938. The D.T.M. examinations are held in December, March and June.
- (2) Two courses for the Diploma in Tropical Hygiene, commencing on the 22nd April, 1937, and the 6th January, 1938. The D.T.H. examinations are held in March and June.

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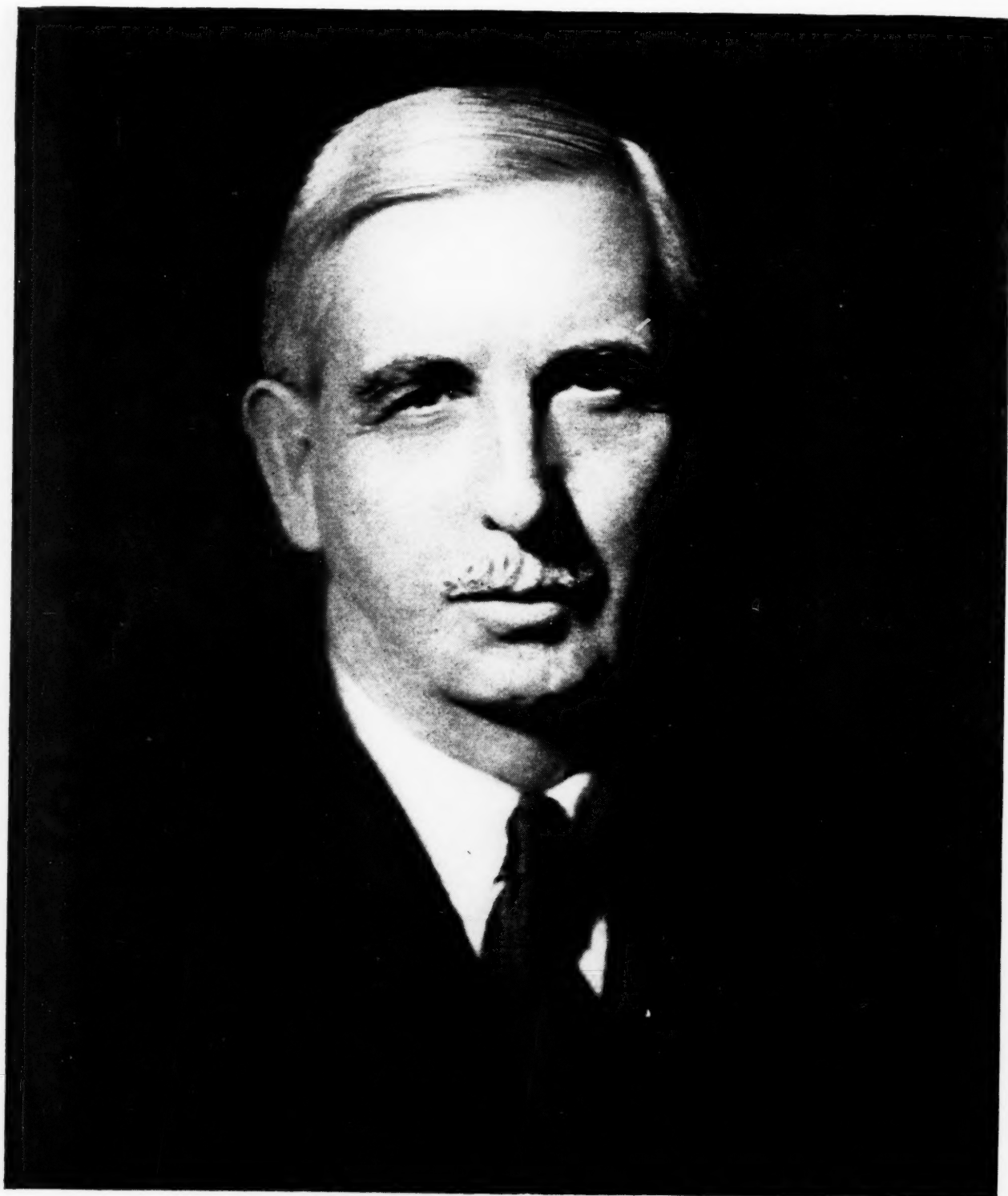
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The Editors have the honour of announcing that
His Majesty King George VI
has graciously consented to become Patron
of the Liverpool School of Tropical Medicine.
His Majesty, as Duke of York, was Honorary
President of the School from 1923 until the
time of his Accession.



Howard Coster, London.

Isabel M. Watson



THE RELATION OF SHADE TO THE NATURAL BREEDING PLACES OF *ANOPHELES COSTALIS* IN MAURITIUS

BY

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(*Received for publication 20 October, 1936*)

Blacklock's (1936) interesting paper on the cultivation of dense shade plants as an anti-malaria measure prompts me to give some account of our experience of this method in Mauritius, where it has been adopted as an anti-*costalis* measure for the past 17 years. A brief reference was made to it in a paper written by me in 1934 (Kirk, 1934); but, as interest will undoubtedly be stimulated by Blacklock's communication, a somewhat fuller account of our experience may be useful to others desirous of trying the method with regard to other species of anophelines in different countries.

Mauritius is a country which for six months of the year is exposed to cyclonic storms. It is a very hilly place, and streams are numerous. During a cyclonic storm, the rainfall is extremely heavy; rivers become torrents, brooks overflow their banks and are liable to scour out a new course altogether, while smaller tributaries and watercourses, which may be dry or reduced to a series of pools during other months of the year, suddenly fill up again and become quite respectable streams.

The sudden flooding to which the rivers and watercourses in the Colony are periodically liable gives rise to the problem of soil erosion. It is by no means a new problem, since the Ordinance still governing the protection of watercourses of the Colony was enacted in 1875, and at that time it consolidated a number of ordinances enacted at various times since 1769. The means whereby such protection has been effected has been by the creation of what are known as 'river reserves,' which are defined in the Ordinance as follows:—"River Reserves" shall mean the ground on either side of a stream between the ordinary edge line thereof or as traced out by stones and boulders and a perpendicular let fall, in the case of a river on a line of 50 feet in length, in the case of a rivulet on a line of 25 feet in length and in the case of a feeder on a line 10 feet in length, projected horizontally to the said edge line; provided always that the river reserves shall include the whole escarpment of any river, rivulet or feeder whatever may be the height or area of such escarpment.'

Rivers are defined as streams in which 50 cubic feet or more of water per minute usually flows. Rivulets have a flow of less than 50 cubic feet and more than 10 per minute; and feeders have a flow of under 10 cubic feet per minute.

Though the land thus demarcated as river reserves remains the property of the riparian owner, he is not allowed to exploit it as he would like. The exploitation of the land is vested in a Board known as the River Reserves Board, consisting of the Director of the Medical and Health Department, the Conservator of Forests and the Inspector-General of Police. From a very early date these reserves have been maintained under trees, and the whole object of the Ordinance is to preserve this growth by prohibiting its removal, unless the riparian owner gives an undertaking to replant suitable trees in the area thus denuded. One weak point in the Ordinance is that no power is given to the Government to require riparian owners of reserves denuded by cyclone or other natural causes to cause them to be replanted. As soon as a suitable opportunity occurs, the Legislative Council will be asked to confer this power on the Government. The Board deals with all questions regarding the maintenance of the reserves, and initiates legal proceedings against those discovered contravening the provisions of the Ordinance, which prescribes adequate penalties for such cases.

The effect of the Ordinance is to ensure a belt of shade of varying depth on each side of the watercourses of the Colony. But it has not been popular with planters, who find that the shade provided by the trees encroaches to a certain extent upon neighbouring sugar-cane plantations. There is always a tendency to nibble away the outer edges of the reserves on this account, if not to eradicate them altogether. This is seen more in the case of reserves running through small holdings than in those traversing large estates. From time to time in the past, the reserves have been assailed, not on account of their deterrent effect on the growth of the neighbouring sugar-canes, but on sanitary grounds. They have been alleged to favour the pollution of the watercourses by providing cover for persons who wanted to ease themselves on the banks, and they have been accused of favouring malaria because of the shelter they afforded to mosquitoes. This, incidentally, was Ross's view. Though it was generally acknowledged that they were a useful means of preventing soil erosion, and though they were thought to exert a favourable influence on the climate in their immediate neighbourhood by conserving a certain amount of moisture in the soil, their sanitary disadvantages were thought to outweigh their advantages, especially when their adverse influence on the growth of sugar-cane was thrown into the balance.

But, fortunately for the Colony, the discovery was made that *Anopheles costalis*, the mosquito principally concerned with the transmission of malaria, did not breed in shaded streams, though it could be found in parts of the same streams open to the sun's rays. It has happened that the felling of trees in reserves has caused a part of the river concerned to be exposed to the direct rays of the sun, and has resulted in the appearance of larvae of *A. costalis* in the exposed part of a river from which they were formerly absent. The first record of this observation is to be found in the report of a committee appointed by the Governor in 1919 to enquire into and report upon the management of river reserves. In

paragraph 16 of this report, we find the following :—' From evidence laid before the Committee it appears that the view was originally held that the destruction of tree growth in river reserves would exercise a beneficial effect since mosquitoes would thereby be deprived of suitable breeding places, owing to the admission of increased light and air. This led to agreements whereby riparian owners were allowed to remove the trees in the reserves and to plant sugar-canes or other trees. Further experience has demonstrated, however, that this view was erroneous. It appears that the conditions created by the removal of belts of trees along the sides of rivers have proved most favourable to the development of mosquito larvae owing to the rapid and luxurious growth of algae and other aquatic plants which takes place in watercourses under the influence of the sun's rays.'

This observation has been made too often over the last 15 years for the occurrence to be mere coincidence, and there is now no doubt that well-maintained river reserves are a valuable weapon in our anti-malarial armament.

It should be noted, however, that this interesting feature in the bionomics of *A. costalis* may be merely a local phenomenon. I am not aware of any similar observation having been made on this species elsewhere, and the species is so widespread that it is unlikely that this peculiarity would have been overlooked had it been evident.

Blacklock's record of another species having the same characteristic as is shown by *A. costalis* in Mauritius points to the desirability of giving more extended trial to shade planting than is apparently the case at present ; but care should be taken that the planting of shade trees along streams does not make the situation worse. There is in Mauritius also *A. funestus*, which, though it does not appear to have any great importance locally in the epidemiology of malaria, is, in some other parts of the world, regarded as a notorious vector. *A. funestus* in Mauritius shows the opposite tendency to *A. costalis* in the selection of waters for egg-laying. It shows a marked preference for waters densely shaded from the sun, or for marshes choked with reeds and other densely growing aquatic vegetation. Fortunately, though fairly widespread in the Colony, this mosquito has a much more restricted range than *A. costalis*, and in actual practice the dilemma of having to choose between two evils, i.e., whether to shade water and risk *A. funestus*, or to leave it exposed to the sun and risk *A. costalis*, has not yet arisen.

Another point which should be noted with regard to the deterrent effect of shade on the choice exercised by *A. costalis* in the selection of water for egg-laying is that what would be considered as light shade appears to be almost as strong a deterrent as dense shade. The photograph accompanying this article shows an appreciable amount of sunlight on the stream ; in fact, it was taken with this object in view. Yet even this slight amount of shading is sufficient to protect the stream.

It may be thought that the method of planting shade trees may be a satisfactory means of treatment of open drains and small streams, but that it cannot be expected to have much effect on broad rivers. This is not so. It is seldom that mosquito larvae are found in midstream; the current itself is a powerful deterrent. Where they are to be found is in the little pools and backwaters occurring along the edges of the river, in places blocked with twigs, leaves and gravel. It is just at these points where the planting of shade trees may be expected to clear up a serious *costalis* nuisance, especially when this is combined with training the edge of the stream and filling in with gravel odd holes holding water.

In Mauritius the jamrosa (*Eugenia jambos* L.) has been found to be the most suitable tree for planting along the water-line of river reserves. Its branches tend to arch over the water and thus provide the desired shade.

In view of these observations, Blacklock's hypothesis that shade-planting might act as a deterrent to certain anophelines by interposing a mechanical barrier between the mosquito and the water appears to me to be scarcely tenable. I think that the explanation is to be found rather in the effect of sunlight on the growth of organisms consumed as food by the larvae of certain species of *Anopheles*. It is possible that the larvae of *A. costalis* and others showing the same heliophilic character require for food organisms which can grow only in water which obtains a minimum amount of exposure to the sun. If the shading accorded by the trees is enough to reduce the exposure below this minimum, these organisms will not grow, and *A. costalis* will not lay its eggs in such water, even though a certain amount of direct sunlight may play upon the water during the course of the day. Comparatively little conclusive work has been published on the subject of the food of mosquito larvae, and it is possible that if the problem were approached from this direction interesting conclusions might follow.

Finally, a word of caution is necessary. It is doubtful if shade-planting by itself will keep a watercourse in a healthy state, especially when it is remembered that other species of *Anopheles* habitually lay their eggs in shaded water. Shaded watercourses will still require the attention of the health authorities with regard to the maintenance of the watercourse free from obstruction, and the training of the banks so as to eliminate small pools and to ensure a brisk current even at the edge of the stream.

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PLATE I

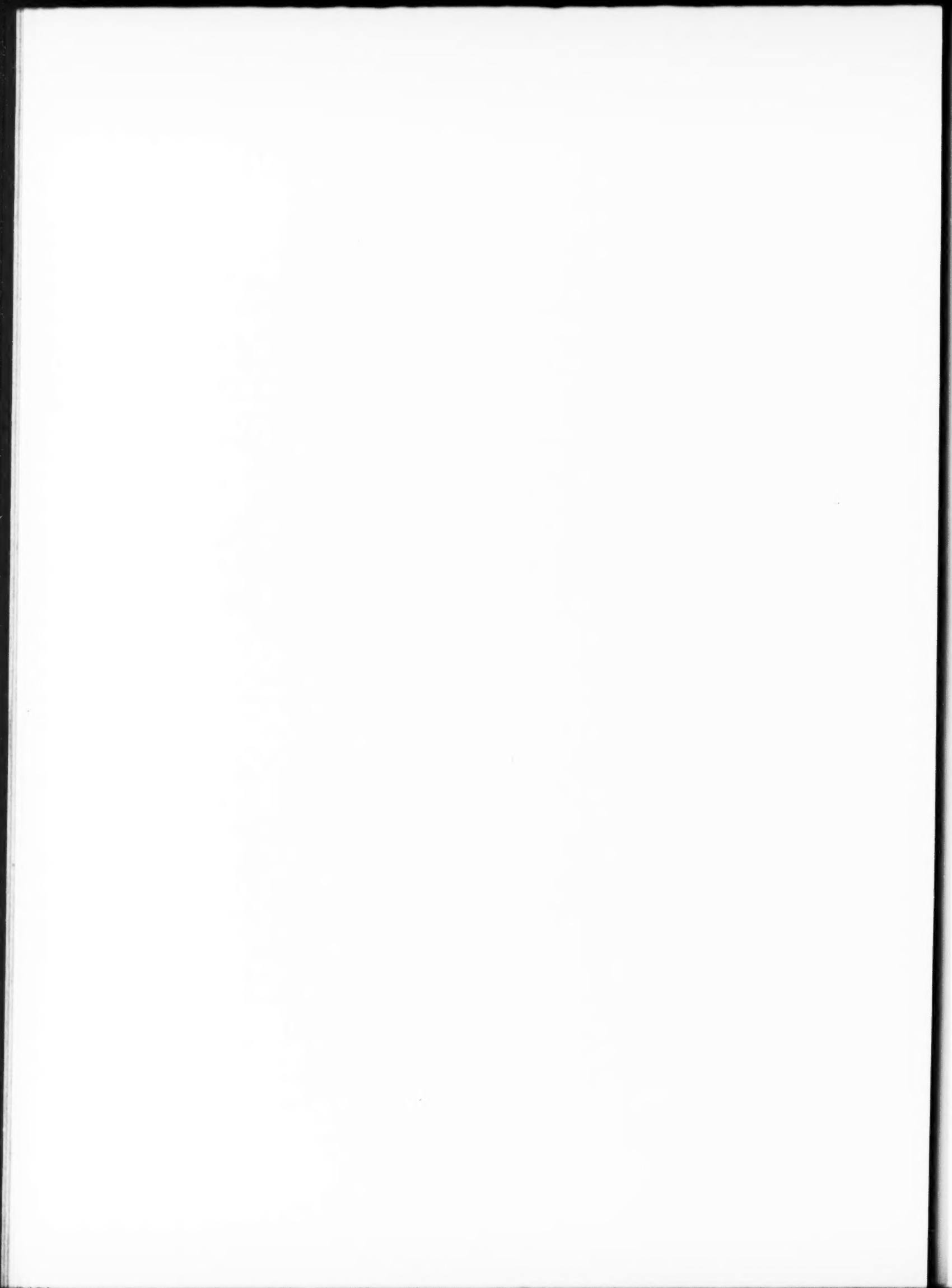
EXPLANATION OF PLATE I

Part of a stream showing how *E. jambos* L. arches over the water. Though the shade is not dense, it is enough to prevent *A. costalis* from egg-laying, even in this untrained part of the stream.



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STUDIES IN THE CHEMOTHERAPY OF MALARIA

THE DISTRIBUTION OF ANTI-MALARIAL DRUGS BETWEEN RED CELLS AND SERUM

BY

J. D. FULTON*

(Received for publication 22 October, 1936)

The question of the distribution of anti-malarial drugs between red cells and serum has been considered of importance with regard to the effect produced in malaria. The relationship of concentration of the drug in red cells to that in serum has been termed the partition coefficient. As the malarial parasite is, at certain stages, attached to or within the red cell, a high partition coefficient would suggest that the drug is brought into and held in greater concentration in contact with the parasite. Again, if trophozoites are to be killed directly, permeability of the red cells for drugs is desirable.

The value of the partition coefficient as determined by different workers has varied with the conditions and the methods employed. A change in value follows replacement of serum by physiological saline as the suspending medium. Concentration is not of importance provided that the relative volumes of the red cells and fluid medium remain constant. Rona and Bloch (1922) find that, with change in the pH value of the medium, salt or base are taken up equally well by the red cells. They consider that adsorption is of chief importance with regard to the value of the partition coefficient, and this view is shared by Gibbs (1928). Shaw (1928) regards absorption by the red cells not as a chemical combination or surface effect, but as the distribution of a solute between two immiscible phases.

Pantschenkow and Kirstner (1928), and also Binet and Fabre (1929), employed the blue fluorescence of quinine sulphate, after extraction of the drug, to compare the amounts present in red blood cells and serum, and found that the partition coefficient has a value greater than unity. Morgenroth (1918), using defibrinated blood and a biological method for the estimation of the drug, as well as Schilling and Boecker (1919), from a study of the precipitation of the potassium mercuric iodide compound of the recovered quinine, reached the same conclusion. Shaw (1928) suspended human and bird red cells in physiological saline and estimated the partition coefficient by an interferometer method, and Rona and Bloch (1921), whose method we have followed, generally found the partition coefficient to be high. Acton and King (1921) and Gibbs (1928), using the same nephelometric method, found the distribution between red cells and the medium to be about equal. On the other hand, Hartmann and Zila (1918), by an optical method, concluded that there was more quinine in the serum.

*Beit Memorial Research Fellow. The work was carried out at the London School of Hygiene and Tropical Medicine.

We thought it worth while to employ the extension of a method used in a previous communication (Fulton, 1936) in some further investigation of this question. A study of the distribution was therefore made in defibrinated human blood for some 20 pure drugs of known constitution and anti-malarial activity. We wished to find if the partition coefficient in this series bore any relation to anti-malarial activity or to chemical constitution of the drug.

The method, with some variations, has the advantage of being a general one for those drugs which can be recovered unchanged from red cells or serum.

Some experiments were first performed with washed red cells suspended in physiological saline to which quinine salt was added in buffered solution. After a fixed time, the mixture was centrifuged and the percentage of red cells and fluid determined. The inhibitory power of known volumes of the fluid in geometrical dilutions, from which the drug had been absorbed by red cells, was compared with that of a standard quinine solution, also diluted in the same geometrical ratio and chosen to cover the range expected. The volume of the unknown solution added was such that it would contain the same amount of drug as the standard, assuming that there had been no adsorption by red cells. The following determination will serve as an example.

Experiment 1. 9.4 c.cm. of washed red cell suspension in physiological saline were well mixed with 1 c.cm. phosphate buffer, pH 7.4, and 2 c.cm. quinine bihydrochloride (4 mgm.), by continuous shaking for 6 minutes. After standing 60 minutes the mixture was centrifuged. Volume of R.B.C.s 2.7 c.cm. (21.8 per cent.). Volume of solution 9.7 c.cm. Therefore, assuming no absorption, 1.3 mgm. of the drug, the amount selected for the control, is contained in 0.808 c.cm. The solutions from which the value of the reaction constant was derived contained

<i>Control</i>	<i>Unknown</i>
30 c.cm. tributyrin	30 c.cm. tributyrin
3 c.cm. buffer	3 c.cm. buffer
1.3 mgm. Q	0.808 c.cm. solution
1 c.cm. human serum 1:1	1 c.cm. human serum 1:1

and corresponding dilutions with factor 3 were also employed.

TABLE I

Control		Unknown				
Quinine in mgm.	K	Volume of solution added	K	Mgm. of quinine in solution in absence of absorption	Quinine found in mgm.	Percentage of quinine absorbed by red cells
0	0.02145	0.808 c.cm. serum absent	0	0	0	
1/81	0.01929	0.808 c.cm. 27 × dil.	0.02155	0.0123	0.00471	61.8
1/27	0.01512	0.808 c.cm. 9 × dil.	0.01876	0.0370	0.0147	60.8
1/9	0.01052	0.808 c.cm. 3 × dil.	0.01392	0.111	0.0493	55.6
1/3	0.00627	0.808 c.cm.	0.00927	0.333	0.151	54.7

The results are shown graphically below. The logarithms of concentration are plotted on the abscissa and the values of K on the ordinate. The controls and the curve for the unknown are indicated. The linear form of the curve indicates that arithmetical decrease of K follows geometrical increase of drug concentration (Rona and Bach, 1920).

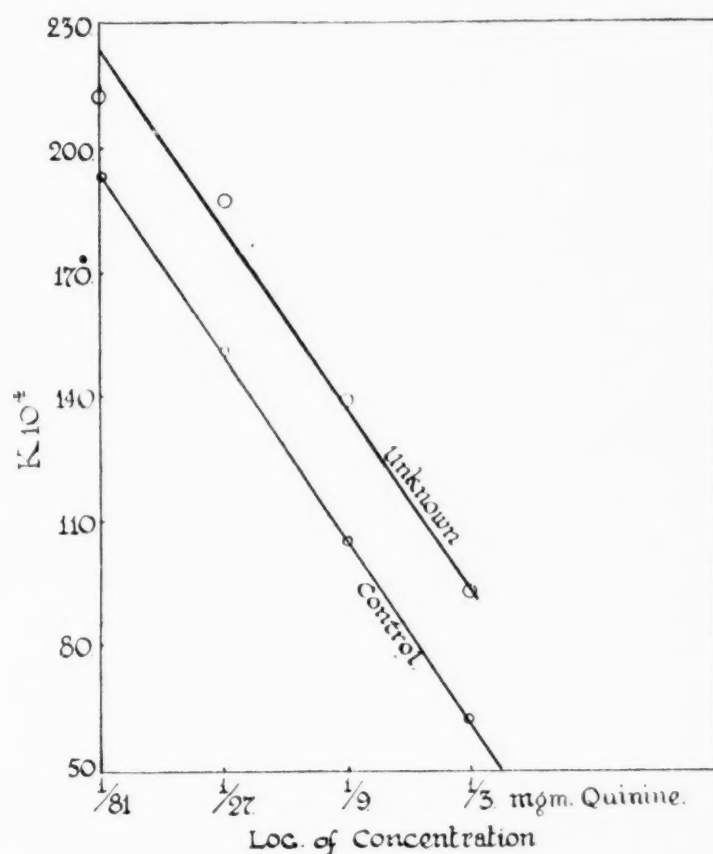
The value for the points plotted on the unknown curve may be calculated from that of K . *Method I.* Let the lowest point on the unknown curve be C_3 , the similar point on the standard curve C_1 and the one above it C_2 . C_3 falls between C_1 and C_2 .

Difference in value of K between C_1 and C_2 is $0.01052 - 0.00627 = 0.0042$.

That between C_1 and C_3 is $0.00927 - 0.00627 = 0.0030$.

0.0042 corresponds to a difference of $\log 3 = 0.4771$.

$\therefore 0.0030$ „ „ „ „ 0.3408 .



$$\log C_1 - \log C_3 = 0.3408$$

$$C_1/C_3 = 2.192$$

$$\therefore C_3 = \frac{0.333}{2.192} = 0.151 \text{ as given.}$$

Method II. A graphical method may be used to find the value of C_3 . Values of K multiplied by a suitable number are plotted as ordinates, and the logarithm of concentration of the drug is multiplied by a factor as abscissa.

The value of C_3 can then be read off directly when that of K is known. The method is not so accurate as that given above.

The results given show that the amounts of quinine bound by red cells agree amongst themselves, and the results were repeatable. The amount taken up by red cells increases, however, with the relative volume of these in the suspension. As stated by Rona and Bloch (1921), it was found that the drug could be recovered from the red cells after being haemolyzed, rendered alkaline and extracted with ether; also, that control cells furnished no lipase-inhibitor when treated in the same way. These authors claim a recovery of 80–90 per cent. of the added drug, and this is justified by our experience. When R.B.C.s and serum respectively are extracted by the same method, inherent errors are presumably the same for each, and tend to neutralize when the partition coefficient is calculated.

The following example will illustrate the method for finding the partition coefficient.

Experiment 2. A. 6.5 c.cm. of defibrinated human blood was mixed with 1 c.cm. of hydroquinine hydrochloride (2 mgm.), the mixture well shaken for 6 minutes, left 1 hour to come to equilibrium and then centrifuged. The volume of red cells was noted and the serum separated. Each was made alkaline with N. caustic soda or ammonia in the case of cupreine and hydrocupreine, to give pH approximately 10. The haemolyzed red cells and serum were then extracted 6 times with an equal or greater volume of redistilled ether, which was collected and removed on the water-bath. The residue was dried *in vacuo* and 1.5 c.cm. N/10 HCl added. The volume was finally made up to 10 c.cm. with distilled water and centrifuged to remove traces of solid material. The solutions of drug thus obtained as hydrochloride were compared with similar standard solutions as in Experiment 1. A duplicate experiment was performed in each case.

B. Volume of red cell suspension, 5.8 c.cm.

Volume of R.B.C.s, 3.1 c.cm.

under the same conditions as A.

The values of K obtained from known amounts of drug in standard volumes were as follows:—without drug, 0.01840; with 1/45 mgm., 1/15 and 1/5 mgm. respectively, 0.00612, 0.00309, 0.00131.

TABLE II

Present in final solution						K	Mgm. of drug in volume used	Total drug recovered in mgm.
A	{	2 c.cm. serum extract	0.00240	0.1046	0.5230
		2 " " " 3 × diluted	0.00454	0.0399	0.5984
		1 " R.B.C.	"	0.00207	0.1258	1.258
		1 " " " 3 × diluted	0.00412	0.0462	1.385
B	{	2 c.cm. serum extract	0.00218	0.1154	0.577
		2 " " " 3 × diluted	0.00483	0.0358	0.531
		1 " R.B.C.	"	0.00207	0.1258	1.258
		1 " " solution.	3 × diluted	0.00440	0.0414	1.242

We see from the above results that the total amount of drug recovered varies from 88.5 to 99 per cent. respectively in the two experiments, but we do not always claim such accuracy. In every experiment quoted, however, more than 80 per cent. of drug was recovered, and in one or two cases was a little above theoretical. The summation of amounts recovered in red cells and serum respectively provided a check on the method.

Many attempts were made to extract the drug by ether in a modified Soxhlet apparatus from solutions similar to the above, with the normal thimble replaced by one of glass, and having a funnel dipping to the bottom of the aqueous suspension, through which ether circulated continuously. It was found satisfactory for serum, but the red cells gave a gelatinous mass which could not be satisfactorily extracted.

In the case of the synthetic quinoline compound R 34, the normal method was not followed, on account of the small inhibitory action of the drug for human serum lipase, and a colorimetric method was therefore employed. In the case of sodium atoxyl and stovarsol, which cannot be extracted with ether, the amount of drug in the suspending physiological saline was estimated directly, and that in the red cells by difference. For hydrocinchonidine, which is insoluble in ether and other solvents except alcohol, Bloor's method (1916) was used.

The results obtained for the partition coefficients are recorded in Table III (see pages 12 and 13).

SUMMARY

The biological method of Rona and Bloch (1921) is suitable for the estimation of small amounts of drug in solution. It is a general method of value in the determination of partition coefficients, provided that the drug can be recovered unchanged. In the case of plasmoguin, for example, oxidation may occur. We have been able to recover more than 80 per cent. of all drugs used. The percentage of drugs bound by the red cells is independent of the drug concentration, provided that other factors remain the same.

From the results obtained we see that :—

1. Laevo and dextro rotatory compounds exhibit no special difference regarding the value of the coefficient, but a group of laevo compounds appear together high on the list.
2. In every case the hydro-compounds have a higher partition coefficient than the corresponding natural alkaloids.
3. The value of the partition coefficient is small in the case of those compounds with long side chains (isobutylhydrocupreine, eucupin, vuzin). These compounds and optochin showed high inhibitory powers for serum lipases. Their readier absorption by serum constituents may explain these facts.
4. With the exception of quinidine, those compounds having an oxygen atom in the quinoline ring have the higher coefficient than the cinchonine-cinchonidine series.

TABLE III

				Partition coefficients		Anti-malarial activity (Giemsa)	Sign of rotation
					Mean		
Hydroquinidine	2.6 3.3	2.9	++ like quinidine	D
Hydroquinine...	3.1 2.6 2.9 2.8	2.8	++	L
Hydrocupreine	2.4 2.5	2.4	++	L
Cupreine	1.9 2.6 2.3	2.3	±	L
Quinine	2.5 2.0 2.5 2.0	2.3	++	L
Atebrin	2.2 2.3	2.2		
R 36	2.4 2.0	2.2		
Hydrocinchonine	1.9 2.2 2.2 2.5	2.2	+ =	D
Hydrocinchonidine	2.4 2.0	2.2	No clinical data	L
Cinchonine	2.2 1.9 1.7 1.4	1.8	+ =	D

TABLE III—(Continued)

				Partition coefficients		Anti-malarial activity (Giemsa)	Sign of rotation
					Mean		
Optochin	1.7 1.6 1.7 1.7	1.7	++ —	
Cinchonidine	1.6 1.4 1.8 1.6	1.6	±	L
R 34	1.6 1.5	1.6		
Quinidine	1.4 1.4	1.4	++	D
Plasmoquin	1.1 1.1 1.6 1.5	1.3		
Isobutylhydrocupreine	...			1.1 1.5 1.2 1.4	1.3		
Eucupin	1.0 0.9	0.9	Little use in malaria	
Atoxyl	0.7 0.8 0.6 0.6	0.7		
Vuzin	0.7 0.7 0.4 0.4	0.5		
Stovarsol	0.4 0.4 0.4 0.4	0.4	++	

5. The partition coefficient is small for the sodium salts atoxyl and stovarsol. The results were, however, obtained for suspensions of red cells in physiological saline by difference.

6. The first six compounds with highest partition coefficient are good anti-malarials. A relationship is not obvious in other cases.

7. For the large majority of the compounds examined the partition coefficient is greater than unity.

ACKNOWLEDGEMENTS. My thanks are again due to Sir Rickard Christophers, for much valuable help in this work.

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ANOPHELES MOSQUITOES IN YUGOSLAVIA AND THEIR ZOOPHILISM

BY

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(*Received for publication 23 October, 1936*)

In Yugoslavia the genus *Anopheles* is represented by five species, namely, *A. bifurcatus*, *A. hyrcanus*, *A. maculipennis*, *A. plumbeus* and *A. superpictus*. Of these, *A. maculipennis* is wide-spread and is the chief vector of malaria; *A. superpictus* is at times found in all malarious districts; *A. bifurcatus* is found in many places, but in small numbers; *A. hyrcanus* is a rare species, except in the rice-fields in the Kocane and Strumica districts, where it is found in abundance; and *A. plumbeus* is a wild species, never found in human dwellings or stables, and therefore of little importance as a vector of malaria.

Anophelism without malaria, such as exists in Italy, is never found in southern Yugoslavia. Dr. Simic (1934) has shown that in Yugoslavia the number of cases of malaria is directly proportional to the number of anopheline mosquitoes. All malaria infections in the first half of the year have a direct connection with those in the second half of the previous year, and vice versa.

Unsuitable stables, and the habit, both of men and of domestic animals, of sleeping at night in the open fields, enable mosquitoes to obtain food easily. In the course of my work, which has extended over a period of eight years in the districts of Skoplje and Prizren, I have noticed that anopheline mosquitoes attack the first person or animal they encounter, and that, provided other conditions are found suitable, they generally remain in the neighbourhood of their first bite. Thus, at Prizren malaria cases are confined to the streets near the flat fields, no cases occurring in the streets at a higher level towards the hills.

Similar findings were also noted in the neighbourhood of Skoplje, where there are a number of suitable stables housing domestic animals of good breed, and where malaria is very prevalent among the villagers.

From the epidemiological standpoint, great numbers of anopheline mosquitoes have no importance in the spreading of malaria if they fail to attack human beings, as is the case in countries where zoophilism prevails. In southern Yugoslavia, however, it is a fact that the greater the number of anophelines, and the longer their season, the more numerous are the cases of malaria.

During the last few years much attention has been given to the races of *A. maculipennis*. This species is, as mentioned above, the most widely distributed in Europe, and is chiefly responsible for carrying malaria parasites. Hackett and Missiroli (1935) record the existence of seven different varieties,

and have made a classification of each, based chiefly on their eggs. These varieties are as follows :—

- (a) *melanoon* (Hackett)
- (b) *messeae* (Falleroni)
- (c) *typicus* (Meigen)
- (d) *atroparvus* (van Thiel)
- (e) *labranchiae* (Falleroni)
- (f) *elutus* (Edwards)
- (g) *subalpinus* (Hackett).

In the opinion of Roubaud (1932), some of these varieties are homophilic in character, others zoophilic. But this cannot be taken as of general application, and the opinions of malariologists differ on the point.

A. maculipennis var. *messeae* does not attack man, and is therefore not a vector of malaria in France, Italy (Lombardy) or Holland; yet in southern Yugoslavia this variety readily and eagerly attacks man, and is responsible for most of the malaria infections of the country.

A campaign organized by Professor Zotta, of Roumania, and many others, to determine the varieties of *A. maculipennis* in Yugoslavia, found only two, viz., *messeae* and *typicus*. In order to obtain more conclusive evidence on this point, therefore, an extensive investigation was undertaken during the malaria season of 1935, on anophelines caught in houses and stables in a number of different villages, with the purpose of identifying the source of the blood which they had engorged. In two and a half months (from July 30th to October 15th) we dissected 812 anopheline mosquitoes and performed 812 haemoprecipitin tests.

I should like here gratefully to acknowledge the exceptional welcome extended to us by the villagers amongst whom we worked. They freely permitted us—though we were complete strangers—to visit their houses in the early mornings, to waken their families, and to disarrange their furniture in our search for mosquitoes; and then, after we had given them so much trouble, they served us with breakfast, for which they would take no payment.

METHOD OF CATCHING THE MOSQUITOES

In order to obtain the greatest possible quantity of fresh blood, all mosquitoes were caught early in the morning, after their abundant night meal. Their stomachs were grossly distended with blood and occupied the whole of the abdominal cavity. (The voracious appetite of these insects is well known: they will suck blood until defaecation begins.) All catches were made by means of test-tubes, from which the mosquitoes were transferred to an entomological gauze cage. Overcrowding or shaking of the test-tubes was carefully avoided, for the distended stomachs easily burst, and the specimens are then useless for experimental purposes.

In order to obtain average results, we chose different houses and stables in different villages, and examined each batch of mosquitoes separately. The

predominant species in all the villages which we searched (Glumovo, Nerezi, Lisicje, Petrovac, Aracinovo, Idrizovo, Marino Selo, etc.) was found to be *A. maculipennis*, except in the village of Orixare, where in the middle of August we found *A. superpictus* in greater abundance.

THE REMOVAL OF THE STOMACH WITH ITS BLOOD CONTENT

The removal of the stomach was not carried out in the manner usual in mosquito dissection (e.g., in a search for oöcysts, etc.), as an incision made over the terminal segments is not large enough to allow the distended stomach to pass through, and, if force is used, the stomach bursts and the blood escapes.

In our technique, the wings and legs are first cut away, and the insect is placed on a slide and fixed by a triangular needle, the abdominal side being exposed upwards. By means of an entomological knife a cut is made over the abdominal segments, beginning at the top and working downwards. The distended stomach then easily comes out through this cut, and, with a few sharp movements of the needle, the stomach is separated from the Malpighian tubules and the oesophagus. The stomach is then placed in a test-tube and broken against the sides, and, by means of a graduated pipette, the spilt blood is washed down with normal saline solution.

BLOOD DILUTIONS

Blood dilutions were made as follows :—

The dilution needed for the tests is 1 in 1,000. It is calculated that the average content of blood in a full stomach amounts to $1/4$ – $1/5$ of a drop. As a rule 1 c.cm. of blood contains 20 drops, so that, when 1 drop of blood is diluted in 10 c.cm., a dilution of 1 in 200 is obtained. From this it follows that $1/4$ or $1/5$ of a drop, diluted in 10 c.cm. of normal saline, gives 1 in 800 or 1 in 1,000, which is the dilution needed for the test.

HAEMOPRECIPITIN TESTS

From the many methods for precipitin tests, Kolmer's technique was chosen. This method consists in adding slowly with a sterile pipette to the blood dilution (e.g., 1 in 1,000) the respective immune serum, in such a way that it will collect at the bottom, with a sharp line of demarcation.

The tubes were not shaken ; they were kept at room-temperature, and the results were read after from 10 to 20 minutes. Any cloudiness that developed after 20 minutes was not taken into account.

The performance of the tests was quite simple, since we were working with a known substance, i.e., blood. The immune sera were highly clear, and were obtained from the Central Institute of Hygiene, Belgrade, the State Sero-therapeutic Institute, Vienna, and the Behring Werke, Berlin.

The titres of immune sera with which we worked were :—

For pig's blood	..	1 in 5,000
„ horse's „	..	1 in 20,000
„ hen's „	..	1 in 5,000 and 1 in 2,000
„ sheep's „	..	1 in 10,000 and 1 in 16,000
„ cow's „	..	1 in 20,000
„ human „	..	1 in 16,000 and 1 in 20,000.

With each new batch of sera, control reactions were made.

Controls

- 1 c.cm. of blood 1 in 1,000 + 0.1 c.cm. of corresponding immune serum.
- 1 c.cm. of blood 1 in 1,000 + 0.1 c.cm. of normal rabbit serum.
- 1 c.cm. of bloodless extract (albumin) + 0.1 c.cm. of immune serum.
- 1 c.cm. of blood dilution 1 in 1,000 + 0.1 c.cm. of normal salt solution.
- 0.1 c.cm. of immune serum + 1 c.cm. of normal salt solution.
- 1 c.cm. of a known blood dilution 1 in 1,000 + 0.1 c.cm. of non-related immune serum.

By these, specific reaction was obtained in test no. 1 only. Those immune sera which showed non-specific or dubious reactions were discarded. The results were read only in daylight, and a black-white slab was used. Any cloudiness that appeared after 25 minutes' standing was not taken into consideration. An attempt was made to avoid all classical sources of error, as mentioned by Nuttall, Kolmer, and others, namely :—

- (a) Opalescent antisera ;
- (b) Weak antisera which might yield false reactions ;
- (c) Too powerful antisera which might react with non-related blood ;
- (d) Too diluted extract of blood, e.g., over 1 in 1,000 ;
- (e) Too much preservative in the antiserum, which would result in slight cloudiness.

All mosquitoes caught in the morning, whether in houses or stables, were tested the same day, because of their quick digestion of blood.

The number of anopheline mosquitoes caught in stables was 502 (*A. maculipennis*, 390 ; *A. superpictus*, 112). Those caught in houses numbered 310 (*A. maculipennis*, 217 ; *A. superpictus*, 93).

Every sample of blood dilution was tested with the six immune sera mentioned above. A certain number of bloods (9 in all) gave no reaction with any of the immune sera, and these were put under the heading of 'blood of some other animals.' With 140 blood samples positive reactions were obtained with 2 or 3 immune sera, e.g., human and horse, or cow and horse, or human, horse

and pig, or other variations of these animals. The results obtained are recorded in the following Tables :—

TABLE I
Number of anophelines caught—812

No. that sucked blood	In houses 310		In stables 502	
	<i>A. maculipennis</i>	<i>A. superpictus</i>	<i>A. maculipennis</i>	<i>A. superpictus</i>
Human only	96	49	61	45
Human and one animal...	25	29	26	9
Human and two or more animals	4	2	5	4
Animal only	78	19	280	44
Two or more animals ...	7	1	23	5
Total	210	100	395	107

TABLE II
Showing the percentages of the blood sucked

	Human blood only		Animal only		Human and animal	
	<i>A. maculipennis</i> Percentage	<i>A. superpictus</i> Percentage	<i>A. maculipennis</i> Percentage	<i>A. superpictus</i> Percentage	<i>A. maculipennis</i> Percentage	<i>A. superpictus</i> Percentage
Caught in houses ...	45.6	48.1	40.8	21.5	13.6	34.4
Caught in stables ...	15.4	42.9	75.7	44.7	7.9	12.4

As can be seen from the above Tables, out of 812 anophelines, 355, or 43.7 per cent., contained human blood in their stomachs. If we analyse this as regards those caught in houses and those caught in stables, we find that those caught in houses clearly show preference for human blood (i.e., they are homophilic in character), while those caught in stables show preference for animals (i.e., they are zoophilic). The high percentage of zoophilism found in stables continues so long as the domestic animals live in them and the anophelines have no chance of biting human beings ; but as soon as this balance is altered they readily show a preference for human blood. This is clearly demonstrated in

the village of Lisicje, where, during four consecutive days (August 4th, 5th, 6th and 7th), 200 *Anopheles* were caught (78 in houses and 122 in stables), and where five field-workers were sleeping out-of-doors in an open field. Here the ratio is only 51.71 in favour of zoophilism in stables; in houses, however, it rises to 55.23 in favour of homophilism. As regards the anophelines caught in this village, they were as follows:—

Caught in houses						<i>A. maculipennis</i>	<i>A. superpictus</i>
Sucked blood	{	Human	33	9
		" + animal...	9	4
		Animal only	21	2
Total	63	15

Caught in stables							
Sucked blood	{	Human	29	7
		" + animal...	13	2
		Animal only	61	10
Total	103	19

DISCUSSION

From the above results it appears that *A. maculipennis* and *A. superpictus* are equally zoophilic as homophilic, whether suitable stable conditions exist in the vicinity or not. The exceptionally high percentage of human blood found in the mosquitoes at Lisicje can be accounted for by the fact that five field-workers were sleeping unprotected in an open field.

It is well known that anopheline mosquitoes (especially *A. maculipennis*) found in houses and stables often, from the influence of microclimatic and other conditions, behave in a curious and not easily explained fashion as regards homophilism and zoophilism. It seems likely, as Roubaud (1932) has pointed out with reference to *A. maculipennis* as a whole, that there exist two physiologically different races—of var. *messeae* at least. One is found in non-malarious regions, and, since it has lost contact with man, its blood-sucking instinct has been directed towards animals. The other frequents malarious regions, and, having preserved its primitive habits, continues to search for human blood.

In view of the instincts of anopheline mosquitoes—especially of *A. maculipennis*—it is our opinion that another instinct exists along with that of satisfying hunger. We have observed that mosquitoes will bite even when so overfed that the stomach is grossly distended, i.e., when hunger cannot possibly be the cause. It would therefore appear that blood-sucking continues until the stomach

is distended to the maximum ; or that small quantities of blood are vomited or defaecated ; or that the whole stomach-content is vomited, and fresh human or animal blood is sucked up. We are inclined to believe that all these three ways are possible ; and, though we have no experimental evidence to support our theory, it is strengthened by the fact that, out of 812 anophelines, we found 140 that had sucked blood from many animals, or from man and one animal, or from man and two or more animals. From our observations it appears that this instinct of *A. maculipennis* and *A. superpictus* to bite even when overfed is most marked in *A. maculipennis*, in a proportion of 9 to 5.

SUMMARY

In southern Yugoslavia (south Serbia) anophelism without malaria is never found. The number of cases of malaria in any one year is directly proportional to the length of the malaria season and to the number of anopheline mosquitoes in that year.

According to our observations, which were made over a period of many years, in the neighbourhood of the towns of Skoplje and Prizren, anophelines attack the first person or animal they come upon, and, provided that other conditions are suitable, usually continue in the neighbourhood of their first bite.

In Europe, *A. maculipennis* has, according to Hackett and Missiroli (1935), seven varieties, of which Professor Zotta and other workers have found only two in south Yugoslavia—namely, var. *messeae* and var. *maculipennis* (*maculipennis* type form). Both these varieties readily attack man, and both transmit malaria, though var. *messeae*, which is also found in France, Italy (Lombardy) and Holland, neither attacks man nor transmits malaria in those countries.

During the anopheline season of 1935, we examined 812 anopheline mosquitoes in many villages, and performed 812 haemoprecipitin tests. Of these, 502 of the mosquitoes were caught in stables (*A. maculipennis*, 395 ; *A. superpictus*, 107), and 310 in houses (*A. maculipennis*, 210 ; *A. superpictus*, 100).

As shown in Table II, *A. maculipennis* caught in stables sucked human blood in 15.4 per cent. of the cases examined ; animal blood in 76.7 per cent. ; and human blood plus animal blood in 7.9 per cent. *A. superpictus* sucked human blood in 42.9 per cent. ; animal blood in 44.7 per cent. ; and human blood plus animal blood in 12.4 per cent. Of those caught in houses, *A. maculipennis* sucked human blood in 45.6 per cent. of the cases examined ; animal blood in 40.8 per cent. ; and human blood plus animal blood in 13.6 per cent. *A. superpictus* sucked human blood in 48.1 per cent. ; animal blood in 21.5 per cent. ; and human blood plus animal blood in 34.4 per cent.

During the anopheline season in which our investigations were carried out, *A. maculipennis* showed a greater tendency to attack human beings than to attack animals, even when caught in stables.

It would therefore appear that the anopheline mosquitoes of southern

Yugoslavia (in this case, *A. maculipennis* and *A. superpictus*) show no leanings towards zoophilism, even under suitable conditions. This may be accounted for by the theory that, as pointed out by Roubaud (1932), there are two distinctive physiological races of anophelines—especially of *A. maculipennis*.

We are of the opinion that, in these mosquitoes, there is, as well as the instinct to feed, an instinct to bite, even when they are actually overfed. A considerable number of mosquitoes show a tendency to suck blood from different kinds of animals or from animals plus man. Of such cases, we found 140, out of a total of 812 anophelines examined.

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A NOTE ON *PHACOCHOEROSTRONGYLUS PRICEI* SCHWARTZ, 1928, AND ON THE MALE OF *OESOPHAGOSTOMUM GOODEYI* DAUBNEY, 1926

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(Received for publication 5 November, 1936)

While in Kalanda, a village in northern Katanga, Belgian Congo, Dr. Richard P. Strong, leader of the 1933-34 Harvard African Expedition for the Study of Onchocerciasis, shot a wart-hog, *Phacochoerus aethiopicus*, which was examined for helminths by the writer.

Associated with numerous specimens of *Oesophagostomum simpsoni* Goodey, 1924, and of *O. goodeyi* Daubney, 1926, in the lumen of the caecum about 20 specimens of a *Phacochoerostrongylus* Schwartz were found, having the following principal dimensions :—

	Female	Male
Length (in millimetres) ...	14	12
Maximum width ...	0.62	0.40
Length of oesophagus ...	0.84-0.88	0.78-0.81
.. .. tail ...	0.95	—
.. .. ovejector ...	0.38	
.. .. vagina ...	0.4	
.. .. spicules...		0.93-0.96
.. .. gubernaculum ...		ca. 0.076

This genus still remains monotypic, being based on *P. pricei* found in a wart-hog that died in the National Zoological Park, Washington, D.C. (Schwartz, 1928). In view of the fact that our specimens are distinctly larger than *P. pricei*, and that the external corona radiata is composed of 16 elements, as contrasted with 10 described by Schwartz, it was at first considered to represent a new species. However, on comparing paratype specimens of *P. pricei*, kindly loaned by Dr. Schwartz, we find that an error had crept into Schwartz's original description, the number of elements in the internal and external corona radiata being 32 and 16 respectively. Our specimens consequently appear to differ from the paratypes only in gross size, and so, in our opinion, do not merit specific distinction.

OESOPHAGOSTOMUM GOODEYI DAUBNEY, 1926

A solitary female represented the material upon which Daubney (1926) originally described this species, secured from a wart-hog in Upper Senegal, French West Africa. The chief diagnostic feature of this species is the salient vulva, which gives the posterior extremity of the female a very distinctive appearance. Fortunately, no more than two species of *Oesophagostomum* parasitized the wart-hog from which our material came. The other species, *O. simpsoni* Goodey, 1924, is a larger and much more robust worm, easy to separate from *O. goodeyi*, whose principal measurements are here tabulated :—

	Male	Female
Length (in millimetres) ...	13-14.5	16-17
Breadth (maximum) ...	0.35	0.56
Length of oesophagus ...	0.54	0.61
" " tail ...	—	0.12
" " spicules ...	1.78-1.95	—
Head to cervical groove ...	0.26	0.29
Length of vagina ...		0.46
Eggs (in utero) ...		ca. 0.068 × 0.040

The differential features of the male are exceedingly difficult to recognize ; had there been no females of *O. goodeyi* present, difficulty would have been experienced in distinguishing the male from such a species as *O. mwanzae*, whose male does not differ very significantly in size.

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OBSERVATIONS ON TICK-TRANSMITTED HUMAN SPIROCHAETOSIS IN PALESTINE

BY

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Since the Great War it has been known that there are cases of relapsing fever in Palestine which cannot be traced to lice. Nicholson (1919) reported a number of cases from soldiers, and from time to time cases occur in people engaged in archaeological investigations in caves, or in people who have slept in or visited caves. A visit to a cave is a factor common to all the cases which we have been able to collect. Some of the cases state that while in the cave they were bitten by an insect rather similar to a bedbug. Nicholson obtained a single tick larva from a site where a soldier had been infected, and this was identified as *Argas persicus*. This tick is widely disseminated in Palestine, and has been suspected, probably wrongly, of transmitting spirochaetosis to man in Persia and in Palestine.

In the present paper we have collected the records of 45 cases of relapsing fever associated with caves, from 1927 up to date; 19 of these cases came under our personal observation, and the others are taken from the records of the Kupath Cholim Hospital in Emek Jezreel. Thirteen cases were traced to a single cave near Kfar Vitkin, a little south of Caesarea; 17 cases contracted the infection in the neighbourhood of Nazareth; 3 cases came from Mishmar Haemek, at the foot of Mount Carmel (2 of these cases were traced to one cave); 1 from upper Galilee; 2 from lower Galilee; and 9 from Emek Jezreel (in the latter 9 cases the history is not complete, and the exact source of the infection was not traced).

Fifteen of the cases occurred in children from the age of $4\frac{1}{2}$ to 11, and the remainder in adults up to the age of 43.

INCUBATION PERIOD

In 13 cases the incubation period, as calculated from the time of being bitten by ticks to the first appearance of symptoms, is known exactly.

On the afternoon of October 14th, 1935, eleven children traced a porcupine to a cave near Kfar Vitkin. Two children stood guard at the entrance of the cave, and nine entered with the object of capturing the porcupine. The children remained two hours in the cave on the additional pretext of carrying out

' scientific archaeological investigations '. All the nine children who entered the cave became infected, the incubation periods being 5 days in one case, 9 days in one case, and 7-8 days in the remainder. The two children who stood guard at the entrance of the cave did not become infected.

On October 29th, at 11 p.m., two medical men entered the cave with the object of determining the transmitting agent; they were accompanied by another medical man, a local farmer and one of the infected children (his last attack terminated 5 days previously), who acted as guide. The three latter stood near the mouth of the cave but did not enter. The two men who entered the cave remained 20 minutes, during which period they were attacked by larvae of *Ornithodoros papillipes*, which swarmed on every part of their body. Although nymphs and adults abounded in the damp dust on the floor of the cave, these did not attempt to attack. After leaving the cave, both men stripped; about 400 bites were counted on one man, 200 on the other, and numerous gorged larvae were collected. Symptoms appeared in the first man at 11 p.m. on November 3rd (at 10 p.m. he enjoyed a meal and felt quite well). In the second case, symptoms appeared between 9 and 10 p.m. on November 5th. Of the two men who stood at the entrance of the cave, one was bitten by 10 larvae and the other by 5; both became infected, the incubation period being 7 days in one case and 8 days in the second. Another man, who was bitten by 12 larvae near the entrance of the cave on October 30th, 1935, did not become infected.

On May 16th, 1933, four farm labourers and a watchman went to sleep in a cave at Kfar Hachores, near Nazareth. Seven days later the four labourers fell ill and were brought to hospital by the watchman, who stated that they had all been bitten by ticks in the cave. The watchman was familiar with the ticks, and, though he often slept in the cave and had been bitten frequently by ticks, he had never been ill. In the other 28 cases, the incubation period is not known.

NUMBER OF ATTACKS

The number of attacks per case varied from 1 to 14. The number of attacks in 33 cases is shown in the following Table :

No. of attacks	1	2	3	4	5	6	8	9	10	11	14
No. of cases...	9	4	8	2	1	3	2	1	1	1	1

The duration of the untreated attacks (the period during which the temperature was raised and spirochaetes appeared in the blood) varied from 3 hours to 4 days. The number of attacks does not appear to have been influenced by the treatment adopted (usually neosalvarsan). It is possibly dependent on the

number of bites by infected ticks, for in the cases under personal observation one case with 400 bites had 11 attacks, one case with 200 bites had 9 attacks, one case with 10 bites had 1 attack, and another case with 5 bites had 1 attack. But there may be other unknown factors, for in the case of one child with numerous bites there was only 1 attack, while in another child with few bites there were 4 attacks. Generally the attacks are fewer and the disease is milder in children than in adults.

SYMPTOMATOLOGY

The symptoms vary from case to case and in different attacks of the same case. The following cases are mentioned briefly, in order to demonstrate the absence of a uniform or even characteristic clinical picture.

CASE NO. 1. ♂ aet. 25. Fell ill on 1.5.28, and the first attack lasted till 4.5.28. Second attack 8.5.28; lasted 1 day. Third attack 18.5.28; lasted 1 day. Fourth attack 22.5.28; lasted 2 days. Fifth attack 6.6.28; lasted 2 days. Sixth attack 23.6.28; lasted 1 day. Spirochaetes found in thick drop. No further attacks.

The case received no specific treatment.

Contrast to the following case:—

CASE NO. 2. ♀ aet. 25. Entered a cave on 21.5.30, together with 4 children. 28.5.30. First attack. Spirochaetes found in thick drop. Two days high fever (temperature up to 39.5° C.), and subsequently irregular low fever up to 37.8° C., till 16.6.30.

- 2.6.30. 0.3 gm. neosalvarsan.
- 5.6.30. 0.45 gm. neosalvarsan.
- 20.6.30. 0.3 gm. neosalvarsan.
- 1.7.30. Second attack lasting 2 days (temperature up to 39.5° C.), stovarsol 0.25 gm. per os 4 times daily up to 18.7.30.
- 27.7.30. Third attack lasting 1 day. 0.3 gm. neosalvarsan.
- 4.8.30. Complained of defective vision.
- 7.8.30. Fourth attack lasting 3 days.
- 10.8.30. 0.36 gm. neosalvarsan.
- 17.8.30. 0.15 gm. neosalvarsan.
- 25.8.30. 0.75 gm. neosalvarsan.
- 6.9.30. An attack lasting 1 day. Spirochaetes found in thick drop. 0.75 gm. neosalvarsan.
- 10.9.30. Left hospital with good vision.

This case received a total of 3.7 gm. neosalvarsan and 18 gm. stovarsol, and ran a more severe course than the above untreated case.

The four children (4–5 years of age) infected at the same time each had one attack lasting 2 days; one child received 0.15 gm. neosalvarsan, and three children received a total of 1.5 gm. stovarsol each per os spread over 5 days.

The following group of four cases is interesting. All four slept in the same cave on May 6th, 1933, and had their first attack on May 13th. Three of the cases had the following treatment :—

0.3 gm. neosalvarsan on second day of attack.
 " " " 5 days after first attack.
 " " " 10 days after first attack.

Of these three cases, one had 1 attack and two had 3 attacks. It is doubtful whether the treatment had any influence on the above three cases, because the fourth case received more neosalvarsan and had 4 attacks. This case received the following treatment :—

13.5.33. 0.3 gm. neosalvarsan.
 18.5.33. 0.3 gm. neosalvarsan.
 23.5.33. 0.6 gm. neosalvarsan.
 6.6.33. 0.45 gm. neosalvarsan. Complained of headache.
 7.6.33. Second attack.
 10.6.33. 0.45 gm. neosalvarsan. Complained of headache.
 27.6.33. Third attack. Liver and spleen enlarged and patient slightly icteric.
 31.7.33. Fourth attack. 0.45 gm. neosalvarsan.

Another case had 3 attacks, of which only the first was treated.

The following group of 13 cases was infected in the same cave and showed considerable clinical variation. Four of the cases, which were under continuous observation, showed an interesting allergic phenomenon, which has not to our knowledge been previously recorded in the literature of tick-borne relapsing fever. In three cases papules appeared on the site of the original bites shortly before or immediately after the termination of each attack; in one case they appeared at the commencement of each attack, became increasingly raised during the attack, and persisted for some days after its termination. The cutaneous manifestations were accompanied by itching, which in one case was sufficiently troublesome to cause insomnia.

CASE NO. 3. ♂ aet. 27. Entered the cave on 29.10.35, and received about 400 bites from larvae.

3.11.35. About 11 p.m. the attack commenced suddenly, with rigor, severe headache and pressure in epigastrium. Thick drops were taken at the commencement of the attack, but no spirochaetes were found. Twelve hours later spirochaetes were found in thick drops, and 0.45 gm. neosalvarsan was given intravenously. Two hours later there was vomiting, followed by diarrhoea. The attack lasted 4 days. Shortly before the fall in temperature, papules accompanied by severe itching appeared on the sites of many of the original tick bites. During the interval between the first and second attack, there was diarrhoea, with mucus and fat in the stool.

17.11.35. Second attack commenced with a rigor (temperature 40° C.), nausea and vomiting. The attack lasted 24 hours and terminated by crisis. No specific treatment was given. Immediately after the attack there was a recrudescence of the papules.

21.11.35. Third attack commenced in the late evening (temperature 38° C.).

22.11.35. Rigor. Temperature 40° C. Nausea and headache. 0.3 neosalvarsan. The attack lasted 50 hours. Again there was a recrudescence of papules on the old sites.

28.11.35. Fourth attack, similar in type and duration to the third attack, with a recrudescence of papules. 0.3 gm. neosalvarsan intravenously.

In the seven subsequent attacks no papules appeared after the fall in temperature.

On 10.12.35, 22.12.35, 9.1.36 and 15.1.36, there were four more attacks similar in type and duration to the third attack. The fifth and sixth were each treated with 0.3 gm. neosalvarsan, but the seventh and eighth attacks were not treated.

17.1.36. The ninth attack commenced with a rigor (temperature 40° C.), lasted 18 hours and terminated by crisis. Spirochaetes were found in thick drops 2 hours before the end of the attack, but were not found in thick drops taken half an hour after the termination of the attack. Two guinea-pigs were inoculated intraperitoneally each with 5 c.cm. of blood taken half an hour after the attack, and did not become infected, although the guinea-pig was found to be extremely susceptible to the spirochaete in question.

31.1.36. Tenth attack, lasted 3-4 hours. Spirochaetes were found in thick drops.

10.2.36. Eleventh attack, characterized by intense headache. Twelve hours after commencement of attack 0.25 gm. solganal B intravenously.

12.2.36. 0.25 gm. solganal B.

In spite of treatment, the attack lasted 50 hours and was similar to previous untreated attacks.

From 14.2.36 to 22.2.36, 0.25 gm. solganal B daily.

22.2.36. Numerous cylinders, both hyaline and granular, and epithelium cells appeared in urine. Albumen 1 per 1,000. After 7 days the urine contained erythrocytes and leucocytes, in addition to cylinders and epithelium of tubules. At the beginning of May a few erythrocytes and cylinders were still present in the urine.

In this case the first attack lasted 4 days, the third to eighth attacks lasted 2 days each, the second and ninth attack lasted from 18 to 24 hours, and the tenth attack only 2-4 hours. The shortest and mildest attacks incidentally were those in which no specific treatment of any kind was given.

CASE NO. 4. ♂ aet. 27. 29.10.35 bitten by 10 larvae.

6.11.35. In the evening rigor (temperature 38° C.), nausea, coughing and dyspnoea.

7.11.35. Received 0.38 gm. neosalvarsan intravenously; but this did not influence the attack, which continued till the morning of 10.11.35. Soon after the fall in temperature papules appeared on the sites of the former bites.

Six weeks after the attack, neuritis appeared in the sciatic nerve, with intense pain in and around the knee, and a notable amount of atrophy of the muscles. The pain was acute for three weeks, after which it subsided, but it was still present at the beginning of May, 1936.

CASE NO. 5. ♂ child aet. 10. Entered cave 14.10.35.

23.10.35. Attack commenced with rigor (temperature 39.6° C.). Two hours after commencement of attack received 0.15 gm. neosalvarsan. Twenty-four hours later temperature fell to normal, and papules appeared on sites of original tick bites.

CASE NO. 6. ♂ aet. 41. Entered cave 29.10.35, and was bitten by about 200 larvae.

30.10.35. Placed an adult tick on left forearm. The tick remained attached for 12 hours, during the whole of which period its presence was not felt. The bite was accompanied by local analgesia, which lasted for 2 days from the moment of puncture.

3.11.35. Three wild ticks (2 ♂♂ and 1 ♀) were placed on the left forearm, in order to study the effect of the bites and particularly the analgesia. The puncture of the skin by one tick was felt distinctly, but the effect was so slight that it might well have been overlooked if special attention had not been paid. Very quickly after the initial puncture, local analgesia set in and remained for 2 days. In the case of the two other ticks the puncture was not felt.

5.11.35. About 10 p.m. an attack of vomiting and fever (temperature 38.9° C.) and severe headache.

6.11.35. Spirochaetes found in thick drops.

At the beginning of the attack the local reactions to the bites of the larvae had not disappeared, and with the commencement of the attack they became red and raised. On squeezing, clear fluid escaped; no spirochaetes were found in the fluid, although they were present in the blood. The attack lasted till November 9th.

7.11.35. 0.3 gm. neosalvarsan intravenously.

19.11.35. Second attack commenced with vomiting, headache and fever (temperature $38.9^{\circ}\text{C}.$), and was similar to the first. The attack terminated 23.11.35. In all attacks the commencement was marked by enlargement of papules on many of the sites of the original tick bites.

20.11.35. 0.3 gm. neosalvarsan.

1.12.35. Third attack, marked by headache and especially by intense pain in the eye-balls. The temperature did not rise above $38^{\circ}\text{C}.$ No treatment. The attack terminated on 5.12.35.

7.12.35. Fourth attack, lasted only 1 day (temperature $38.7^{\circ}\text{C}.$).

11.12.35. Patient felt quite well. Three ticks (*Ornithodoros papillipes*), which had fed on 3.11.35 on an infected rat, were allowed to feed on the left forearm. The ticks completed their feed within half an hour; one puncture was distinctly felt for a short time, after which there was local analgesia; in the other two ticks the analgesia was instantaneous.

13.12.35. Fifth attack commenced at 3.30 p.m., with nausea, inability to stand, and headache.

14.12.35. Spirochaetes were found in thick drops, but scrapings from the puncture wounds made on the previous day by the three infected ticks 40 hours after feeding were negative. The attack terminated at 2 a.m. on November 15th. A few hours later thick drops were taken and were found negative.

16.12.35. Sixth attack commenced 8.30 p.m. and lasted about 18 hours. 0.2 gm. metallic bismuth in the form of a suspension injected intramuscularly.

29.12.35. Seventh attack, lasted 1 day. 0.2 gm. metallic bismuth injected intramuscularly.

1.1.36. Eighth attack commenced at midnight and terminated on 3.1.36 at 2 a.m.

5.1.36. Noticed on looking down microscope that the field was full of apparently dancing particles.

9.1.36. Iridocyclitis diagnosed by Dr. A. Feigenbaum of the Hadassah Hospital. Subsequently, small capillary haemorrhages in the retina and oedema of the papillae were noted in both eyes.

21.1.36. Ninth attack, lasted a little more than 24 hours. Temperature $37.9^{\circ}\text{C}.$ Cerebrospinal fluid, taken at commencement of attack, examined and found normal. 1 c.cm. cerebrospinal fluid was inoculated intraperitoneally into two guinea-pigs, which did not develop spirochaetosis. 5 c.cm. blood inoculated intraperitoneally into each of two guinea-pigs. Both became infected.

Subsequently, opacities developed in the vitreous of both eyes, particularly in the right. Vision fell from $5/4$ to $5/8$, but by the beginning of April, 1936, vision was restored to $5/4$, although slight opacities in the vitreous still persisted.

21.1.36. During attack the blood picture was as follows:—

R.C. 4,590,000 (the normal for this patient before his illness was 5,500,000).

Hg. 85 per cent.

Leucocytes: 8,300.

Neutrophils 60 per cent.

Eosinophils 6 per cent.

Monocytes 4 per cent.

Lymphocytes 30 per cent.

An eosinophilia of 6 per cent. was normal for this case before his illness, although he had no helminthiasis or skin infection.

22.1.36. During the crisis, 0.25 gm. solganal B inoculated intravenously. The injection was repeated every second day till a total of 3 gm. had been administered. A noticeable eosinophilia (up to 9 per cent.) developed, probably as the result of the injections of solganal B; but towards the end of the treatment this had subsided to the normal 6 per cent. After the third injection of solganal B, a marked leucopenia (4,200) and a slight anaemia (4,030,000) developed, but both the red and the white count approximated to normal two weeks later (4,810,000 and 6,800).

A few hours after the first six injections a number of papules became prominent on the sites of previous bites, particularly on the bites of three ticks which were known to be

infected. These papules reappeared from time to time after the treatment had ceased and in the absence of other clinical manifestations. By the middle of May, 1936, the sites of six of the original bites were still marked by a faint brown staining.

Of the nine children who had been infected in the same cave as cases no. 3-6, one had a single attack, five had 2 attacks, two had 3, and one had 5. The number and duration of attacks bear no relationship to the amount of treatment with neosalvarsan (e.g., a child of the age of 10 had a single attack, treated with 0.15 gm. neosalvarsan; another had 5 attacks, the first treated with 0.15 gm. neosalvarsan and the other four each with 0.3 gm. neosalvarsan).

One case deserves special mention, for it indicates that spirochaetes may remain over a long period in human beings.

CASE NO. 7. ♂ aet. 21. 26.6.35. Entered hospital after 5 untreated attacks, each of which lasted 1-2 days. Spirochaetes were found in thick drops. This patient had 5 attacks in the early summer of 1934, and stated that he had not visited any caves in the interval.

Summing up the symptomatology of the cases, it appears that there is nothing characteristic of the disease beyond temperature and the presence of spirochaetes in the blood during an attack. The first attack starts suddenly without any premonitory symptoms. The attacks last from a few hours to 4 days, but in one exceptional case a single attack lasted 19 days, in spite of two injections of neosalvarsan (0.3 and 0.45 gm.).

The attacks may be accompanied by intense pain in the eyes, headache, nausea, vomiting, dyspnoea and pains in the joints, but all these signs may be absent in individual attacks. The disease is much milder in children than in adults, but pains in the joints, particularly in the metacarpo-phalangeal joints, are commoner in children. The variations in the clinical manifestations were such that it is impossible to draw a typical clinical picture, and there is certainly no characteristic temperature-curve. The intervals between attacks varied from 2 days to 6 weeks, but in one case, the history of which is not absolutely certain, there was an interval of about a year between two attacks.

A slight enlargement of the spleen and liver and a hardly noticeable icterus occurred in one case. Complications occurred in only three cases of our series, neuritis of the sciatic nerve in one case and eye complications in two cases. In one of the latter, which was particularly studied, there were iridocyclitis, capillary haemorrhages in the retina, oedema of the papillae and opacities in the vitreous.

In four cases particularly studied, an allergic phenomenon—viz., reactivation of the tick bites—was noted. This phenomenon is probably more common than this number indicates.

Spirochaetes are few in the blood, and should therefore be looked for in thick drops. The blood changes are not very marked. In cases in which they were studied, there was a slight leucocytosis (up to 14,000) in some attacks, and a slight anaemia at the end of the disease (a deficiency of up to 1,000,000 red cells per c.mm.).

TREATMENT

The cases were treated by different physicians, and treatment was therefore not uniform. Incidentally, two untreated cases fared better than many of the neosalvarsan-treated cases. Neosalvarsan in doses of 0.3 to 0.75 gm. for adults, administered during the attacks or in intervals between attacks, appeared to have no effect on the clinical course or on the interval between attacks. It appears that this particular spirochaete has a considerable degree of natural resistance to neosalvarsan in therapeutic doses.

In two cases the serum of convalescents was tested for prophylactic and curative properties in guinea-pigs, with a negative result; doses up to 1.5 c.cm. of serum inoculated intraperitoneally had no effect whatever on the course of the infection in guinea-pigs inoculated with homologous strains of spirochaetes.

The relapsing fever studied resembles the Central Asiatic and Persian types, rather than that of the known Mediterranean types.

TRANSMISSION

The ticks collected in the cave where 13 cases were infected were provisionally identified as *Ornithodoros papillipes*, and this diagnosis was confirmed by Mr. Cecil Warburton, F.R.S., to whom specimens were sent for identification.

Nicolle and Anderson (1928) infected guinea-pigs with ticks (*O. papillipes*) sent from Bokhara, and named the spirochaete which they transmitted *Sp. sogdianum*. This appears to be the correct name for the tick-transmitted spirochaete of Palestine.

Moskwin (1929) found that *O. papillipes* transmits spirochaetosis in Usbekistan. He noted that this tick does not pass coxal fluid during the act of feeding, and that transmission is therefore by bite. He failed to infect susceptible animals by the inoculation of coxal fluid. He also failed to transmit the infection by larvae of infected mothers.

In our cases it was proved by direct observation that four human beings were infected by the bites of wild larvae, and it is therefore obvious that larvae from infected mothers are capable of transmitting the spirochaetes, i.e., the infection passes through the egg.

The following transmission experiments were carried out:—

A. With larvae from infected mothers.

(1). *O. papillipes* ♀ fed on rat on 5.3.36.

13.3.36. Spirochaetes found in blood of rat.

15.4.36. The same tick fed on guinea-pig.

21.4.36. Spirochaetes found in blood of guinea-pig.

4.5.36. Twelve larvae from the above tick (hatched from eggs laid between 5.4.36 and 14.4.36) fed on a clean guinea-pig. Result negative.

(2). 5.3.36. *O. papillipes* ♀ fed on rat.

10.3.36. Spirochaetes found in blood.

15.4.36. Same tick fed on guinea-pig.

19.4.36. Spirochaetes found in blood.

6.5.36. Twenty larvae from this tick fed on a clean guinea-pig. Result negative. These larvae hatched from eggs laid from 2.4.36 to 14.4.36.

(3). 2 ♀♀ (wild) fed on 30.10.35 on child during attack of fever.

21.1.36. Fed on rat recovered from spirochaetosis and negative for spirochaetes since 19.11.35. Result negative.

8.3.36. One hundred and twenty-seven larvae from the above ticks (hatched from eggs laid between 7.2.36 and 23.2.36) fed on a clean rat.

20.3.36. Spirochaetes found in blood.

For all the above experiments the ticks were kept at a constant temperature of 30° C.

It is therefore evident, from both field observations and laboratory experiments, that some larvae of infected mothers are capable of transmitting the disease ; but many individual larvae are incapable of transmitting, either because spirochaetes are not present in the salivary glands, or because a number of eggs from infected mothers are clean. *O. papillipes* is so far the only known vector of human tick fever which is capable of transmitting in the larval stage.

B. Transmission experiments with nymphs.

(4). 16.4.36. One hundred and nine first stage nymphs, developed from the larvae used in experiment 3, fed on a clean rat.

11.5.36. Spirochaetes found in blood.

(5). 5.11.35. Nine wild nymphs (second and third stage) fed on clean rat.

10.11.35. Spirochaetes found in blood.

It will be seen from the above experiments that all stages of *O. papillipes* are capable of transmitting by bite.

FEEDING HABITS AND EFFECTS OF BITE

It was found by observation on wild and laboratory-raised larvae that the act of feeding lasts, as a rule, from 15 to 30 minutes. Approximately the same period suffices for nymphs and adults, although there are exceptions. Four adults placed on an infected child did not complete their feed within 2 hours, and in one instance previously noted an adult remained attached for 12 hours. Soon after attachment of all stages of the tick, there is an area of redness round the puncture wound, which spreads till it is about half a centimetre or even more in diameter. The inner two-thirds of this area quickly change to a bluish colour. Analgesia commences in most cases immediately after puncture, and extends to the whole area of the local reaction. The analgesia produced by most of the bites lasts for about 2 days, but in a few individual bites continued up to 7 days. The duration of the local reaction varies in different individuals and in different bites from 5 or 6 days till much longer. The site of 6 bites, of which 3 occurred on December 12th, 1935, and 3 on January 1st, 1936, were still evident by the end of May, 1936.

O. papillipes, as Moskwin rightly observed, does not pass faeces or coxal fluid while attached to its host. A few minutes after it has left its host it passes a copious amount of coxal fluid. If the ticks are disturbed after completing their feed, the excretion of coxal fluid may be delayed up to an hour.

TRANSMISSION FROM COXAL FLUID

As previously stated, transmission from coxal fluid does not occur under natural circumstances. Moskwin failed to transmit spirochaetosis by the inoculation of coxal fluid into animals. The probable explanation for this failure is that the coxal fluid is not constantly infected with spirochaetes, or that spirochaetes are present in it only at a certain stage of the infection.

5.3.36. *O. papillipes* ♀ fed on a rat. After the completion of the feed it was placed on its back in a clean glass vessel. After about 15 minutes, fluid exuded from both glands. The fluid was collected in a capillary and inoculated intraperitoneally into a clean guinea-pig.

10.3.36. Spirochaetes were found in the blood.

A parallel experiment, carried out with another tick of the same batch, gave a negative result.

ENTRY OF SPIROCHAETES INTO THE VERTEBRATE HOST

Moskwin was of the opinion that after 12 days no spirochaetes are found in *O. papillipes*, although the tick is still infective 127 days after the infecting feed. This author found spirochaetes in ticks sectioned and stained by Levaditi's method from 3 to 12 days after an infecting feed, but no traces of spirochaetes were found after 15, 20, 25, 30 and 65 days. He suggested that the spirochaetes become metamorphosed into granules and minute vesicles which he detected in the salivary glands.

The following experiments were carried out :—

12.12.35. Three wild ticks (2 ♂♂ and 1 ♀) fed on a human being during an interval between two attacks. (These ticks had previously fed on a heavily infected rat on 3.11.35.) The puncture wounds from the human volunteer were scraped and the smears stained with Giemsa. About half of the material was examined and 7 spirochaetes were found.

9.1.36. The same ticks as in the previous experiment were fed on a clean rat. The puncture wounds from the rat were scraped, smears made and stained with Giemsa. Only a part of the material was examined and 4 spirochaetes were found.

These experiments show clearly that spirochaetes are present in the salivary glands of *O. papillipes* and are directly inoculated as such into a wound at least 67 days after an infecting feed.

SUMMARY AND CONCLUSION

Cases of tick-borne relapsing fever from Palestine are described.

The incubation period is from 5 to 9 days.

The number of attacks in different cases varied from 1 to 14.

The duration of attacks varied from 3 hours to 4 days. (In one exceptional case an attack lasted 19 days.)

The attacks did not show a typical temperature-curve.

The only factors common to all attacks were the presence of spirochaetes in the blood and a temperature of which the peak varied from 37.8 to 40° C. Some attacks were accompanied by nausea, vomiting, headache and pain in the eyes and joints. Complications were noted in three cases—neuritis of the sciatic nerve in one, and ocular changes in two.

The disease was not influenced by neosalvarsan in doses of 0.3 to 0.75 gm., administered during or between attacks, and *Spirochaeta sogdianum*, the causative organism, is considered to be naturally arsenic-resistant.

Ornithodoros papillipes Bir. was found to be the transmitting agent. The bite of *O. papillipes* is accompanied by local analgesia which lasts from 2 to 7 days. An allergic phenomenon, consisting of the appearance of papules on the site of original tick bites, was observed in four cases—in one case at the commencement of attacks, and in three cases towards their termination.

O. papillipes does not pass coxal fluid during the act of feeding, and transmission is by bite.

The coxal fluid is infective.

Spirochaetes were demonstrated in puncture wounds made by infected ticks in man and a rat, 39 and 67 days after the infecting feed.

All stages of *O. papillipes* transmit by bite. This is the first recorded instance of transmission by larvae.

The spirochaete passes from infected female ticks through the eggs, but not all larvae are infective.

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DESCRIPTION OF A POLYCEPHALIC CESTODE LARVA FROM *MASTOMYS ERYTHROLEUCUS*, AND ITS PROBABLE IDENTITY

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(Received for publication 25 November, 1936)

A polycephalic cestode larva (fig. 1), found attached by a peduncle to the peritoneal surface of the spleen of a *Mastomys erythroleucus* (a multimammate rat), was sent to us for identification by Dr. A. J. Walker, who is stationed at our laboratory in Sierra Leone.

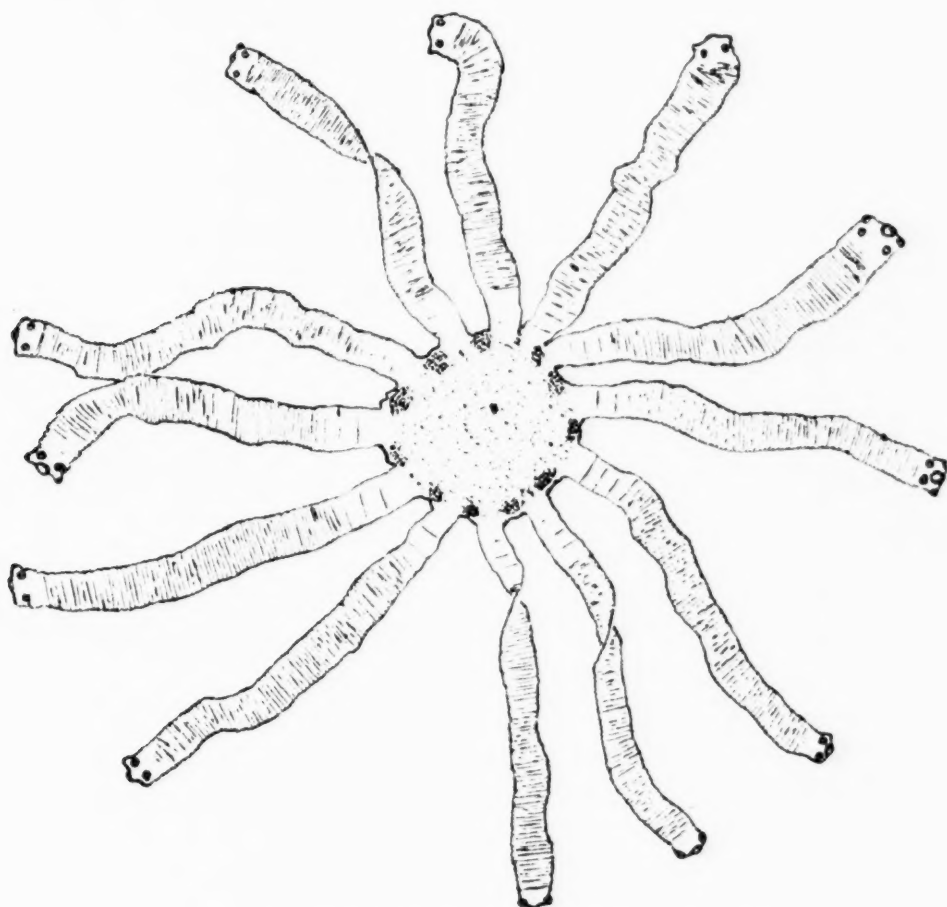


FIG. 1. Polycephalic cestode larva. ($\times 2$.)

DESCRIPTION

This larval form consists of a very small, central, globular, transparent bladder, measuring 10 mm. in diameter. Radiating from this central bladder were 12 segmented strobila (fig. 1), each of which bore at its distal extremity

4 suckers and a double crown of hooks. Each strobila measured from 20 mm. to 22 mm. in length, and had a breadth of from 2.2 mm. to 2.5 mm.

These young strobila radiated with regularity round the cyst, occupying positions identical with the hours 1 to 12 on the face of a clock—that is, they radiated outwards in the same plane, and at almost regular intervals. The head, situated at the extremity of each strobila, measured approximately 550μ by 620μ in breadth, and 2 mm. in length. Each bore 4 prominent suckers and a typical crown of 44 hooks—22 large and 22 small (fig. 2). The large hooks measure from 380μ to 400μ in length. The handle is nearly straight, being slightly sinuous along the dorsal border, with a tendency for the distal extremity to turn dorsally. The ventral and dorsal borders, seen from the lateral aspect, are approximately parallel. The guard projects ventrally at right angles to the handle. The proximal part is cylindrical, the terminal part cone-shaped; the blade is moderately curved. The small hooks measure from 220μ to 240μ in length. The handle is short, with a slight tendency to turn dorsally at the

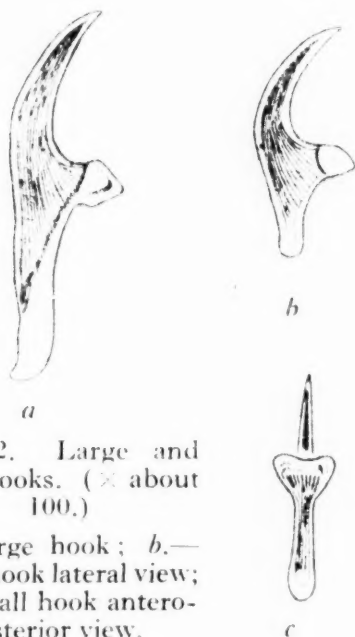


FIG. 2. Large and small hooks. (\times about 100.)

a.—Large hook; *b.*—Small hook lateral view; *c.*—Small hook antero-posterior view.

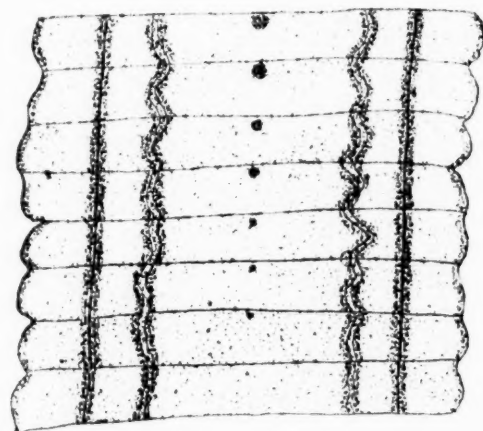


FIG. 3. Median portion of 'neck.' (\times about 20.)

distal extremity. The guard projects at right angles to the dorsal outline of the handle, and is distinctly bifid when viewed from the antero-posterior aspect. The blade is strongly curved. It will be noted that the hooks in our larval form differ very slightly in shape from those of *C. fasciolaris*, as figured by Hall (1919), but we regard this slight difference as a variation normal within the limits of a single species. A *camera lucida* drawing of the hooks is shown in fig. 2.

COMPARISON OF THE ABOVE FORM WITH KNOWN COENURI

The following is a list of those different species of the genus *Taenia* whose larval form is a *Coenurus*, together with the number and size, in microns, of the large and small hooks in each species:—

	No. of hooks	Size of large hooks (in microns)	Size of small hooks (in microns)
(a) The species from Sierra Leone (multimammate rat)	44	390-400	220-240
(b) <i>Multiceps multiceps</i> Zeder, 1800 (sheep)	44-64	150-170	90-130
(c) .. <i>gaigeri</i> Hall, 1919 (goats)	56-64	160-180	115-150
(d) .. <i>serialis</i> Gerv., 1847 (rabbit)	52-64	135-175	78-120
(e) .. <i>spalacis</i> Dies., 1863 (coyapu)	Species bears a single circle of hooks only		
(f) .. <i>lemuris</i> Cobbold, 1861 (lemur)	64	127	—
(g) .. <i>polytuberculosis</i> Mégn., 1879 (jerboa)	—	70	50
(h) .. <i>glomeratus</i> Raillet and Henry, 1915 (gerbil)	32	96-105	58-65
(i) <i>packii</i> Christenson, 1929 (hare)	—	140-150	96-100
(j) .. sp. Baylis, 1932 (man)	30-34	140-155	110-115

It will be obvious that the species from Sierra Leone differs markedly from all the others.

It should be noted that listed coenuri not mentioned above are considered by Hall (1910) to be synonyms of either (b) or (d).

The name *Coenurus* is given to those larval forms of the genus *Taenia* (*Multiceps*) which consist of one bladder and many heads, the typical forms being *Coenurus cerebralis* and *C. serialis*. Variations are known. Thus Cobbold (1861), in his description of *C. lemuris*, calls attention to the fact that, in his species, several pedunculated coenuri were fused together, presenting the appearance of numerous lobulated colonies. In other words, large numbers of coenuri had become fused together into a pedunculated lobular mass. The same condition appears to obtain in *M. polytuberculosis*.

Gaiger (1907), describing a *Coenurus* from the goat in India (*M. gaigeri*), states that 'there was a very distinct tendency in most cysts towards budding off of daughter cysts, and, although actual separation of a daughter cyst was not seen, in one case there was a distinct neck dividing off a portion of the parent cyst. Internal budding was complete, and many of the cysts were floating free.' Hall (1919), dealing with Gaiger's species, found that 'a striking feature of the species . . . is the extraordinary ease with which the heads detach from the bladder wall, and so come to lie free in the internal fluid.' The so-called daughter cysts of Gaiger, therefore, proved to be scoleces which had become detached from the wall of the cyst and thus come to lie in the cystic fluid. This, of course, is most unusual, and does not occur in other species of *Coenurus*.

According to Braun, the scoleces in *Cysticercus* (? *Coenurus*) *botryoides* Boettcher, 1862 (not *C. botryoides* Heller, 1874), fall off into the lumen of the bladder; this is, morphologically, an almost identical condition to that found in *C. gaigeri*.

COMPARISON OF OUR FORM WITH OTHER POLYCEPHALIC CYSTICERCI

Polycephalic cysticerci differ from coenuri in that, whilst in both forms there is one bladder and many heads, it would appear that in typical coenuri the scoleces consist of small nodules closely applied to the cystic wall internally, and that, as a rule, each scolex is invaginated. Even if the scoleces were evaginated, they would still remain almost sessile *on the internal wall of the bladder*. Setti (1897) described from the Egyptian gerbil a polycephalic cyst (*Cysticercus taeniae-brauni*) which resembled a *Coenurus* in consisting of one bladder and many heads, but differed from a *Coenurus* in having the scoleces developed externally and not internally. Linstow (1902) named this form *Cysticercus taeniae-brauni*. In *T. brauni* there are from 60 to 64 hooks, the large ones measuring 130μ to 140μ , the small ones 85μ to 90μ . This is believed to be the larval form of *Taenia crassiceps* Rudolphi, 1810. In consisting of a single bladder and numerous scoleces, this species resembles the one which we are here recording; but, amongst other things, it differs markedly from our species in the size and shape of the hooks.

Rudolphi in 1819 described, under the name *Cysticercus longicollis*, a peculiar larval form from *Mus arvalis*. In 1897 Bott not only figured this species, but gave a lengthy description of it. His figures would suggest that a large number of cysticerci had developed together at one place, giving the appearance of a number of vesicles differing widely in size, and more or less fused together. The condition may be abnormal, but it occurs commonly. The species bears a double crown of from 24 to 28 hooks, the large ones measuring 190μ and the small ones 140μ .

The following is a list of species of the genus *Taenia* with hooks about the size of those found in the Sierra Leone form :—

	No. of hooks	Size of large hooks (in microns)	Size of small hooks (in microns)
<i>T. taeniaeformis</i> (Bloch, 1780)	26-52	380-420	250-270
<i>T. laticollis</i> Rud., 1819	38-60	380-420	150-182
<i>T. macrocystis</i> (Dies., 1850)	60-74	320-365	180-200
<i>T. parva</i> Baer, 1925	44	361	228
<i>T. infantis</i> Bacigalupo, 1922	36-40	410	260
? = <i>T. taeniaeformis</i>			

Hall (1919) considers that *T. laticollis* is a synonym of *T. taeniaeformis*. All the above, except *T. infantis*, are from the cat family, and all have comparatively large hooks. The hooks in our species most closely conform to those of

T. taeniaeformis; further, the presence of a segmented strobila measuring from 20 mm. to 22 mm. is not merely suggestive, but practically diagnostic, of *T. taeniaeformis*. In no other species of which the authors are aware, is there in any larval tapeworm a short segmented strobila behind the head. For these reasons we consider our form to be *Cysticercus fasciolaris*. We can only speculate as to the reason why there are 12 typical strobila arising from a single bladder. It may be that, due to some abnormality, the bladders of a dozen young typical cysticerci have fused together during the early developmental stages, or alternately that, instead of the usual single invagination, and for some unknown reason, more than one invagination occurred in a single bladder. We would call attention to the abnormal position in which the cyst occurred: it was found attached by a peduncle to the peritoneal surface of the spleen.

Villot (1883), in his well-known monograph, described, amongst a number of other larval forms, a *Polycercus* in which several scoleces are formed within a single blastogene (bladder). This is to all intents and purposes a *Coenurus*, from which it differs only in that the scoleces become detached from the wall and fall into the cystic fluid (as in *Coenurus gaigeri*). It is true that up to the present, as far as the writers are aware, no polycephalic *C. fasciolaris* has ever been observed, but we would draw attention to the following series of developmental forms which are known to exist:—

1. *Cysticercus*, consisting of one bladder and one head, as in *C. bovis*.
2. *Monocercus*, a cysticercus in which the scolex eventually comes to lie inside the cyst, as in the larval stage of some species of *Anomotaenia*.
3. A typical *Coenurus*, like *C. cerebralis*, in which there is one bladder and several scoleces, all of which are produced internally (not externally), and which adhere to the wall and do not drop into the cystic fluid.
4. *Coenurus gaigeri*, like no. 3 above, but in which the scoleces may detach from the internal germinal epithelium and come to lie free in the cystic fluid. It would appear that this coenurus does not differ, morphologically, from a *Polycercus*.
5. *Polycercus*, a single bladder in which there are several scoleces formed within the cyst, as in the larval stage of *T. nilotica* found in certain earthworms. These scoleces, each of which is really a cysticercoid, detach from the wall and drop into the cystic fluid. In this, as in no. 3 and no. 4 above, the scoleces are produced internally, that is, inside the cyst.
6. (a) Our parasite, in which several scoleces are formed externally and do not become detached.
- (b) As far as we are aware, the only other polycephalic cyst in which the scoleces develop externally is the form described by Setti in 1897, namely *Cysticercus taeniae-brauni*.

DIAGNOSIS

The number, form and size of the hooks, together with the segmented strobila behind the head, leave us no option but to conclude that each of these strobila is indistinguishable from *C. fasciolaris*.

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DISSOCIATION CONSTANTS AND SOLUBILITIES OF BASES OF ANTI-MALARIAL COMPOUNDS*

I.—QUININE ; II.—ATEBRIN

BY

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(Received for publication 8 December, 1936)

Under anti-malarial compounds may be included the cinchona alkaloids and derivatives and various new synthetic compounds. All the four common cinchona alkaloids, though differing in degree of effectiveness, have anti-malarial properties. Many of the cinchona derivatives likewise have anti-malarial properties, in some cases (e.g., hydroquinine) apparently as powerful as quinine. Among new synthetic compounds are plasmochin and atebrin, selected by their discoverers on account of their special effectiveness and other suitable properties from a large number of compounds of the same type synthesized (Schulemann, 1932; Mauss and Mietzsch, 1933). Other less-well-known compounds of similar constitution are in use as anti-malarial drugs in certain countries. Besides those compounds actually selected for use as drugs, a very considerable number of others are now known to be in varying degree effective, but the reasons for their effectiveness or ineffectiveness are unknown.

In accordance with usual chemical nomenclature, all these compounds, including the natural alkaloids, are substitution derivatives of quinoline or acridine, stress being laid upon such derivation in relation to the possession of anti-malarial properties. They may, however, also be regarded, though somewhat unconventionally, as substitutions of the quinoline or acridine nucleus in certain alkyl or, in the case of the natural alkaloids, cyclic amines. This double nature gives a rather special character to these compounds. Whilst the inclusion of the quinoline or acridine nucleus may be regarded as giving to them an alkaloidal type of structure, in that they are thus basic compounds in which at least one N atom forms part of a cyclic system (Schmidt, 1932), their amine nature conferring strong basic characters would equally appear to be fundamental to the type of compound with which we are dealing; and the possession of basic properties seems, therefore, unlikely to be a mere coincidence or an unimportant part of the make-up of an anti-malarial drug. In the account given by Schulemann (1932) of the researches of himself and his co-workers leading to the discovery of plasmochin, there is very strikingly brought forward the fact that, until a basic component was included in the molecule, no success was achieved.

*Work carried out as Leverhulme Fellow under the Medical Research Council.

Whatever part the quinoline or acridine portion of the molecule may play, it would certainly appear to be the case that no quinoline or acridine compound not possessing an amino group gives any indication of possessing anti-malarial properties.

In such considerations there is a very obvious suggestion that the possession of fairly strong basic groups may be a mechanism to enable these compounds to combine in the body, e.g., with the proteins of the tissues or parasite. It might be expected in this case that the degree of basicity would have some importance. If the basic groups are too weak they will not function as such at the pH of the body; if too strong, the compounds would be completely dissociated and so be practically in the position of strong alkalies, and might on this account fail to meet the requirements for an anti-malarial drug.

It seemed worth while from such a point of view to give some attention to the dissociation constants in this class of compound. These give all information regarding the basicity of basic compounds, and if any relation exists between therapeutic effect and degree of basicity this should be made evident from a study of the dissociation constants covering a sufficient range of compounds.

So far, however, only the constants for the cinchona alkaloids and some of their derivatives are known, as determined chiefly by Kolthoff (1925). The first constants for the four cinchona alkaloids range from a pK value of 5.43-5.97 (equivalent approximately to pH 8.0-8.5), whereas very many organic bases are either considerably stronger, i.e., a pK value of the order of 4, or much weaker, i.e., a pK value of 9 or more. The data, however, do not cover a sufficiently wide field to enable any definite conclusions to be drawn. Of the constants for the many new synthetic compounds, nothing is known. It was decided, therefore, to determine the constants for a number of representative substances of this type.

Closely connected with the dissociation of organic bases is the solubility of the undissociated base. Very low solubility of the undissociated base, judging from experiences with atebirin, might in itself conceivably be a reason for the ineffectiveness of some compounds. Whilst therefore determining the dissociation constants, it was decided also to work out the solubilities of the bases. This again has been done by Kolthoff for the cinchona alkaloids, but no information appears to be available regarding the new synthetic compounds.

In the present paper are given the results obtained for quinine and for atebirin. The determinations for quinine were undertaken in part to gain experience, and may be regarded as of interest chiefly in the confirmation which they give of the previous results of Kolthoff, to which they closely approximate. The determinations for atebirin, so far as known, have been made for the first time.

Whilst the accuracy of the determinations is not final, since for such a purpose the activities of the salts dealt with require to be taken into account, the degree of accuracy attained is probably sufficient for the purpose for which

the observations were undertaken; and as the electrometric data have been given subsequent correction may be possible.

CONSTITUTION

The N atom in the quinoline and acridine ring in each case forms a possible dissociative point in the molecule of the compound (see fig. 1). Dissociation from this source may be expected, however, to be weak, in accordance with the weak character of quinoline or acridine as bases. In quinine the constant from this source is of the order of 10^{-10} . The constant for quinoline, as determined by Kolthoff, is $10^{-9.5}$. Acridines are weaker bases than pyridines or quinolines (Schmidt, 1932).

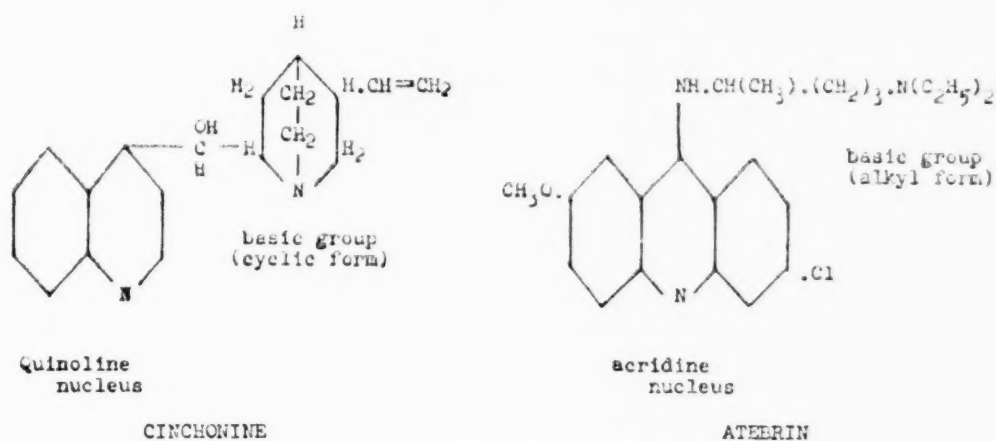


FIG. 1

Further dissociation-points in the molecule are formed by the amine system.

In the synthetic anti-malarial drugs, the amine group has an alkyl form with a terminal N atom, and one at the ring end of the chain, as in atebrin or plasmochin.

In the cinchona alkaloids and their derivatives, there is present, instead of the alkyl basic chain, a second carbon ring with an N atom. Essentially, however, the arrangement seems to be the same, viz., a system possessing a dissociation centre or centres over and above that in the quinoline or acridine portion of the molecule.

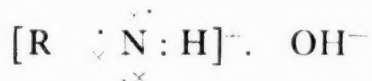
The constants derived from the amine system appear to be more powerful than those in the quinoline or acridine ring, that for quinine being of the order of 10^{-6} and the two determined for atebrin 10^{-4} and $10^{-6.5}$.

DISSOCIATION

The structural formula of the compounds as usually given is that of the unhydrated molecule. I have not been able to find in the literature any direct reference to the mechanism of hydration and dissociation of quinine and bases of this type; but the following treatment, in accordance with recent views of

the dissociation of organic bases generally, seems most suited to deal with the facts.

According to Sidgwick (1927), in the unhydrated molecule of amine bases the N atoms concerned are in the trivalent form with three bonds occupied. On hydration an H atom is regarded as taken up, with loss of an electron and the formation of a positively charged ion in electrovalent linkage with OH^- as shown, where dots represent electrons of the N atom and crosses those of atoms in covalent linkage.



The steps in dissociation of the base would, following such a view, be $\text{B} + \text{H}_2\text{O} \rightleftharpoons \text{BH}^+ \cdot \text{OH}^-$ and $\text{BH}^+ \cdot \text{OH}^- + \text{H}_2\text{O} \rightleftharpoons \text{B}(\text{H})_2^{++} \cdot (\text{OH}^-)_2$, where B is the unhydrated molecule as usually depicted, and where linkage of the two forms BH^+ and $\text{B}(\text{H})_2^{++}$ might be with either OH^- or some other anion, e.g., Cl^- .

In the text, the three forms of the molecule, as set out above, are given as B, B^+ and B^{++} , it being immaterial what view be held of the nature of the substances if the existence of (1) undissociated base, B, (2) the monovalent ion, B^+ , and (3) the divalent ion B^{++} , be assumed. B is considered to be unhydrated base, since on the view adopted any fraction hydrated becomes *ipso facto* dissociated. B^+ may be regarded as existing in the two forms, dissociated base, $\text{B}^+ \cdot \text{OH}^-$, and basic salt, $\text{B}^+ \cdot \text{Cl}^-$. B^{++} for practical purposes is the neutral salt, $\text{B}^{++} \cdot (\text{Cl}^-)_2$.

Strictly, the dissociation constant should be K, as in the equation on the right below, and not k as derived from the usual mass equation on the left.

$$\frac{[\text{B}^+][\text{OH}^-]}{[\text{BOH}]} = k \qquad \frac{[\text{B}][\text{H}^+]}{[\text{B}^+]} = K$$

Nevertheless, since in practice K is readily derived from k, or vice versa

($K = \frac{k_w}{k}$), it seemed undesirable to alter the usual mass formulae, which are

therefore given in the section on formulation, as if the steps of dissociation of a diacid base were $\text{B}(\text{OH})_2 \rightleftharpoons \text{BOH}^+ \cdot \text{OH}^-$ and $\text{BOH}^+ \rightleftharpoons \text{B}^{++} \cdot \text{OH}^-$. No ambiguity should arise if the terms B, B^+ and B^{++} are taken in the sense given above.

METHODS

Many methods have been used to determine the dissociation constants of acids and bases. We are most concerned with those having application to dibasic or diacid compounds, since all anti-malarial compounds so far known are diacidic.

The constants for many organic acids have been determined by conductivity methods. Bredig (1894) especially has determined by conductivity methods the constants for a number of organic bases. The second constants, even of dibasic acids, are, however, determined with difficulty in this way (Gane and Ingold, 1928; Vogel, 1929; Jeffery and Vogel, 1934).

The constants, especially of very weak bases, have in many cases been determined through hydrolysis of their salts, either by determining the free acid by its effect in sugar inversion and in other ways, or by indicator or potentiometric determination of the pH of solutions of the salts (Denham, 1908; Enklaar, 1912; Pugh, 1929). For determination of the constants of aniline derivatives, Farmer and Warth (1904) have used extraction with benzene to determine the free base liberated.

In determining the constants of dibasic acids, McCoy (1908) and Chandler (1908) used an ether-extraction method by means of which the concentration of the free undissociated acid is ascertained after shaking mixtures of the acid and its salts with ether.

Titration methods have been very commonly used. Hastings and van Slyke (1922) determined the second and third constants for citric acid by electrometric titration. They ascertained from the ends of the titration curve the values of k_1 and k_3 , and, making allowance for the amount of NaOH required for these dissociations in the middle of the curve, they calculated k_2 . Recent determinations of the constants of dibasic acids have been made by Gane and Ingold (1928), using potentiometric measurements in the course of neutralization with NaOH. A very complete mathematical treatment of the method has been given by Auerbach and Smolczyk (1924). Kolthoff (1925) has determined the constants for a large number of natural alkaloids by titration methods with indicators, as shown by the pH at the half neutralization point.

For titration of alkaloidal bases, the H electrode has been considered unreliable, owing to the reduction of the base at the platinized surface (Kolthoff, 1925). With certain bases, however, Gilbert (1924) and Prideaux and Gilbert (1927) have obtained satisfactory results. The quinhydrone electrode is not suitable in general for titration of alkaloidal bases, as it can only be used in acid or weakly alkaline mixtures.

The determinations now made have been based on potentiometric observations of the pH under various conditions, using the glass electrode. The results appear to be reliable and consistent, except where noted, if due attention be paid to technique. The apparatus used was that supplied as Morton's, by the Cambridge Scientific Instrument Co. But in place of the combined calomel electrode and mixture chamber it was found more convenient to use a separate calomel electrode with a long arm furnished with a small movable glass plug (as supplied by the same firm). The arm dips into the solution to be tested, in which the bulb is also immersed, the vessel containing the fluid being raised and lowered by a rack and pinion.

Where titration alone has not sufficed, this has been combined with additional methods, such as the ether-extraction method or the solubility method described. The method of determining the pH is applicable not only to all forms of titration, but also to hydrolysis, to ether-extraction and to solubility methods.

Unless otherwise stated, all concentrations given are molecular. The temperature, unless otherwise stated, was 20° C. k_w for this temperature has been taken as 14.06. The water used was thrice distilled in pyrex, giving a pH of 5.7. Freshly boiled or 'CO₂-free' water was used in solubility determinations.

FORMULAE USED

Dissociation of a divalent base may be regarded as proceeding in two steps, as follows, square brackets indicating concentrations (see, however, previous remarks) :—

$$\frac{[\text{OH}^-] \cdot [\text{BOH}^+]}{[\text{B(OH)}_2]} = k_{b_1} \quad (1)$$

$$\frac{[\text{OH}^-] \cdot [\text{B}^{++}]}{[\text{BOH}^+]} = k_{b_2} \quad (2)$$

where k_{b_1} and k_{b_2} are the first and second dissociation constants respectively. For simplicity, since only basic constants are being dealt with, these are subsequently referred to as k_1 and k_2 . Also, for reasons already given, $[\text{B}]$, $[\text{B}^+]$ and $[\text{B}^{++}]$ are used for $[\text{B(OH)}_2]$, $[\text{BOH}^+]$ and $[\text{B}^{++}]$ in the above formulae. Further to avoid ambiguity, due to the medical use of the terms acid and neutral salt in the case of quinine in another sense than the physical one, B^+Cl^- and $\text{B}^{++}(\text{Cl}^-)_2$ will be referred to as the monosalt and disalt respectively.

Equations (1) and (2) giving the values for the constants in the notation used in the text are conveniently used in the $-\log$ equations (3) and (4) (Clark, 1928).

$$\text{pk}_1 = \text{pOH} - \log \frac{[\text{B}^+]}{[\text{B}]} \quad (3)$$

$$\text{pk}_2 = \text{pOH} - \log \frac{[\text{B}^{++}]}{[\text{B}^+]} \quad (4)$$

and in the special cases where $[\text{B}] = [\text{B}^+]$ and $[\text{B}^+] = [\text{B}^{++}]$

$$\text{pk}_1 = \text{pOH} \quad \text{pk}_2 = \text{pOH} \quad (5)$$

When NaOH is added to a solution of an alkaloidal salt, it may be assumed that an equivalent of base, either as $[\text{B}]$ or $[\text{B}^+]$, is liberated.* In the case

*This is probably not strictly true, as the point of complete liberation of all base is approached.

of a monoacidic compound $[B]$ would be equal to N , where N is $[NaOH]$ added, or more correctly $N - [OH^-]$; B^+ is similarly $M - N$, or more correctly $M - N + [OH^-]$, where M is the original concentration of the salt. The same applies to each dissociation of a diacid base where the effects of k_1 and k_2 do not overlap and the monosalt and disalt are respectively being titrated. The dissociation curve is given by plotting the observed pH against a . If

$$\frac{[\text{dissociated}]}{[\text{undissociated}]} = \frac{M - N + [OH^-]}{N - [OH^-]} = f, \quad a = \frac{f}{f + 1} \quad (6)$$

Thus, neglecting the small value of $[OH^-]$ if M be 0.01 and $[NaOH]$ added be 0.001, $f = 0.009/0.001 = 9$ and $a = 9/9 + 1 = 0.90$.

Where k_1 and k_2 are nearer than 10^4 , allowance has to be made for the fact that base may be present in each of the forms $[B]$, $[B^+.OH^-]$ and $[B^+.Cl^-]$. When, therefore, the disalt is being titrated, the first two require in each case two equivalents of $NaOH$ to bring about their formation, and the last only one equivalent. Since, therefore, $N = 2[B] + 2[B^+.OH^-] + [B^+.Cl^-]$ and $[B^+] = [B^+.Cl^-] + [B^+.OH^-]$ and $[B^+.OH^-] = [OH^-]$

$$[B^+] = N - 2[B] - [OH^-] \quad (7)$$

$$[B^+] = M - [B] - [B^+] \quad (8)$$

Where $[B]$ is not known, but k_1 is, an allowance for $[B]$ in k_2 titration can be made from the following formula derived from equations (3) and (7) :—

$$[B] = \frac{N - [OH^-]}{\text{antilog}(pOH - pk_1) + 2} \quad (9)$$

Where the base is present in absence of its salts, the following applies :—

$$k_1 = \frac{[OH^-]^2}{C - [OH^-]} \quad (10)$$

where C = total concentration of the base. k_2 is suppressed, except in very low concentrations. For various reasons the method is of little critical value.

The following derivations from equations (1) and (2) apply to hydrolysis of the salts (Findlay, 1933; Kolthoff and Furman, 1926).

For the disalt

$$pH = 7 - \frac{1}{2}pk_2 - \frac{1}{2}\log C \quad (11)$$

For the monosalt

$$pH = 14 - \frac{1}{2}(pk_1 + pk_2) \quad (12)$$

For the solubility product equation (1) gives

$$[B^+] \times [OH^-] = k_1 \times [B]_{\text{sat.}} = L \quad (13)$$

i.e., under conditions of saturation of the undissociated base, both sides of the equation give L , the solubility product.

ACKNOWLEDGEMENTS

I wish to convey my thanks to Messrs. Howards and Sons, to whom I am greatly indebted for the gift of various pure cinchona alkaloids prepared by them. I am also greatly indebted to the firm of Bayer and Co., who at the suggestion of Professor W. Schulemann have very kindly provided me with atebirin bihydrochloride and atebirin base, without which the work on atebirin could not have been carried out.

To Dr. C. G. Pope, of the Wellcome Physiological Laboratories, Major H. C. Brown, C.I.E., I.M.S., of the Wellcome Research Laboratories, and Dr. R. S. Twigg, of the London School of Hygiene and Tropical Medicine, I am indebted for help and advice regarding glass electrode apparatus and technique. I also wish to thank Dr. J. D. Fulton, Beit Fellow for Medical Research, for much kind help in chemical and other matters.

To Professor W. Schulemann I am indebted not only for help in obtaining material for my studies, but also for his stimulus and encouragement in work on the physico-chemical properties of anti-malarial drugs, to which it is hoped that the observations now being undertaken may form a contribution. Lastly, I wish to thank Dr. F. Mietzsch, of Elberfeld, for useful information about atebirin and for help in other ways.

I.—QUININE

CONSTITUTION

The constitutional formula for quinine adopted by von Oettingen (1933), after Rohde and Antonaz (1907) (see also Rabe *et al.*, 1931), is as follows :—

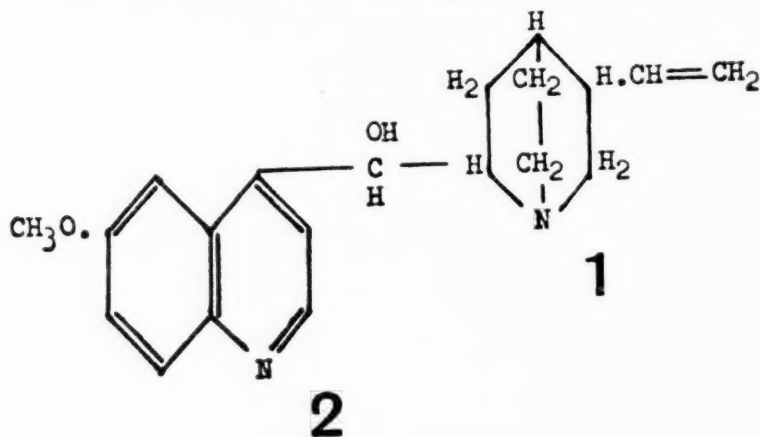


FIG. 2

There are two N atoms capable of giving rise to dissociation constants, viz., at (1) and (2). According to Fischl and Schlossberger (1933), the first, which is the stronger, relates to the N in the quinuclidine (piperidine) nucleus, the second, which is much weaker, to the N in the quinoline ring (see also Colson and Darzens, 1893; Salamonsen, 1895; Berthelot and Gaudechon, 1903; Veley, 1908b).

MATERIALS AND TECHNIQUE

The hydrochloride (mw. with 2 molecules water = 396.5), which was chiefly used in the determinations, was Howards' *purissimus for research*. Where the alkaloid was used, this was a very pure sample kindly supplied to me by the above firm. The bihydrochloride was an ordinary pure sample of the drug.

The method of determining the constants has been by titration of the monosalt with NaOH and HCl respectively for the k_1 and k_2 constant. As results with the glass electrode were a little different from those given by Kolthoff obtained with indicators, I have also made some indicator determinations. The relation of the constants to hydrolysis of the salts is also noted.

Determinations of the solubility of the amorphous and crystalline form of the alkaloid have been made at different temperatures on mixtures of the hydrochloride and NaOH. To determine the saturation point in the latter case, a tiny pledget of microcrystals was allowed to sink to the bottom of the tube or flask, and the reading was made when, at various times up to a week, it could be determined that these had disappeared (-), or increased (+). A (\pm) reading was given when, after an appropriate time, there was no change. Freshly boiled distilled water was used in making all mixtures.

The results of other workers relating to the dissociation and solubility of quinine have already been given by Kolthoff and need not be further considered here (see Manz, 1904 ; Velej, 1909 ; Barratt, 1910 ; Arnall, 1920 ; Schoorl, 1922 ; McGill, 1922 ; Masucci and Moffat, 1923).

For brevity, the contractions Q, Q⁺ and Q⁺⁺ are used in the text and Tables, and the salts are given as QHCl and Q2HCl respectively.

FIRST DISSOCIATION CONSTANT

Titration. In Table I are given data obtained with mixtures of the hydrochloride and NaOH. [Hydrochloride] and [NaOH] are given as M and N respectively (equations 3 and 6).

TABLE I

Determination of k_1 from titration of the hydrochloride with NaOH[illegible]

Eight separate determinations of mixtures of $[\text{salt}] = [\text{base}] = 0.001$ gave $\text{pk}_1 = 5.70$, with a variation of 5.69–5.71 only. The mean of all the series is also 5.70. The constant has therefore been taken as $k_1 = 5.70$ at 20°C . The dissociation curve follows closely the theoretical line for $\text{pk} = 5.70$ except at the two extremes (fig. 3).

Determinations have also been made with cresol red as indicator and are given below. The concentrations are as in Table I. The figures with the glass electrode are given for comparison. It is evident that the glass electrode pH values are throughout about 0.2 greater than those with the indicator, though against standard buffers the readings were identical in the two cases within a maximum departure of 0.02. A corresponding decrease is therefore shown in pk . The value obtained is therefore very close to that of Kolthoff (5.97 at 16°), allowing for the indicator difference and temperature.

α	pH		pk_1	
	Glass electrode	Cresol red	Glass electrode	Cresol red
0.206	8.94	8.72	5.70	5.92
0.30	8.69	8.50	5.71	5.93
0.40	8.56	8.35	5.67	5.89
0.50	8.36	8.20	5.70	5.86
0.60	8.19	8.01	5.70	5.87
0.70	8.03	7.81	5.66	5.88
0.80	7.81	7.65	5.65	5.81
	Mean		5.68	5.88

pH of solutions of the hydrochloride. The mean of a number of determinations gave the pH of the hydrochloride in 0.05 concentration as 6.23, and in 0.001 concentration as 6.20. As calculated from the two constants as here given and equation (12), the pH for both these concentrations should be 6.29. Very similar figures were obtained by Kolthoff. Rather than the pH of the salt it would perhaps have been desirable to determine accurately the turning point by titration.

pH of solutions of the alkaloid. The following are determinations for mixtures of equal concentration of QHCl and NaOH.

Concentration	pH	Concentration	pH
0.0015	9.23	0.0005	9.25
0.0014	9.25	0.0002	8.97
0.001	9.24	0.0001	8.45

The figures are the means of a number of determinations, the individual values obtained being very variable. From equation (10) the pH of 0.001 solution should be 9.7, but so high a figure has never been obtained. The method is of no critical value in determining the constant, but it is of interest to note the very high pH value of even very weak solutions of the alkaloid.

SECOND DISSOCIATION CONSTANT

Titration. In Table II are given the results obtained from mixtures of the hydrochloride and HCl. The concentrations of QHCl and HCl are given as M and N' respectively. $[\text{OH}^-]$ is negligible in the calculation of Q^{++} , Q^+ and α (equations 4 and 6).

TABLE II

Showing calculation of k_2 for quinine from titration of the hydrochloride with HCl

M-N	N'	pH	pOH	α	$\frac{[\text{Q}^{++}]}{[\text{Q}^+]}$	$\log \frac{[\text{Q}^{++}]}{[\text{Q}^+]}$	pk_2
0.009	0.001	5.12	8.94	0.10	0.111	-0.95	9.89
0.008	0.002	4.78	9.28	0.20	0.25	-0.60	9.88
0.007	0.003	4.56	9.50	0.30	0.43	-0.37	9.87
0.006	0.004	4.38	9.68	0.40	0.67	-0.17	9.85
0.005	0.005	4.21	9.85	0.50	1.00	0.0	9.85
0.004	0.006	4.05	10.01	0.60	1.50	0.17	9.84
0.003	0.007	3.88	10.18	0.70	2.33	0.37	9.81
0.002	0.008	3.69	10.37	0.80	4.00	0.60	9.77
0.001	0.009	3.46	10.60	0.90	9.00	0.95	9.65
Mean							9.82

A value of 9.85 is given at half neutralization point and as the mean between values at $\alpha = 0.40 - 0.60$. pk_2 has therefore been taken as 9.85. As there is a progressive change, observations were made with half neutralization mixtures of different concentrations. The constant calculated on these was

Concentration Q^{++} and Q^+	Constant
0.01	9.84
0.005	9.85
0.002	9.83
0.001	9.80
0.0005	9.76

Hence it would seem that the apparent pK increases with concentration, which would be the case if activity of the divalent Q^{2+} was decreased disproportionately to that of the monovalent Q^+ ion with increasing concentration. A graph for the second dissociation curve is given in fig. 3.

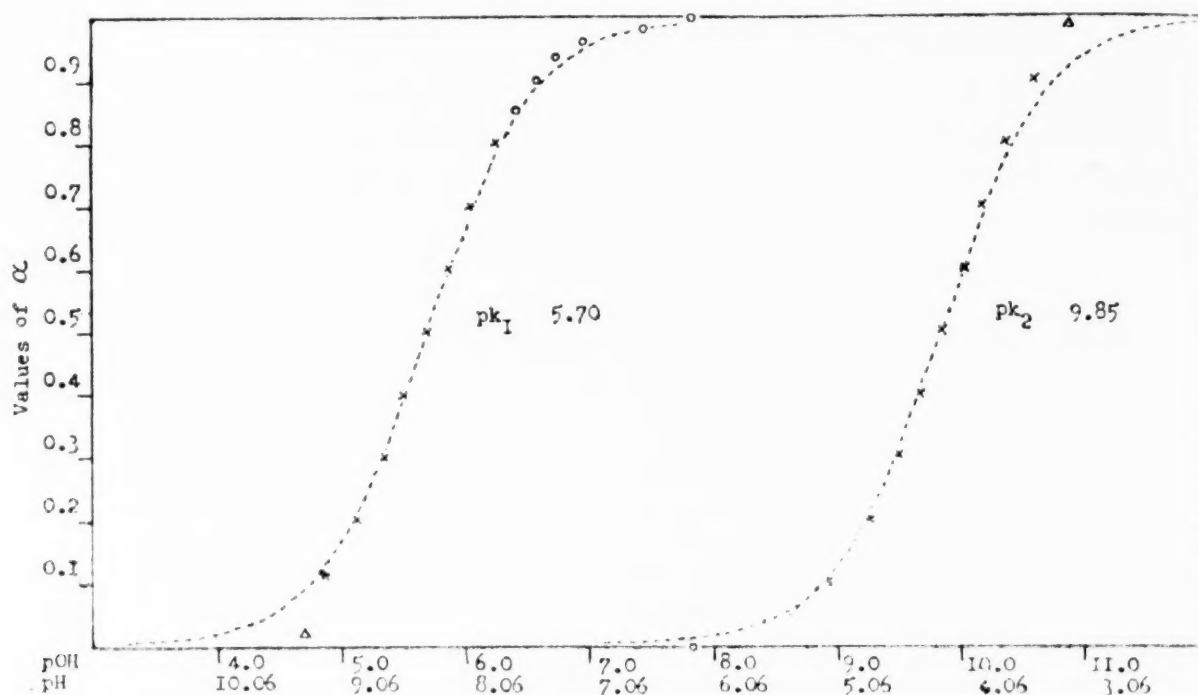


FIG. 3

Showing dissociation curves for quinine. The dotted lines give the theoretical curves for $pK_1 = 5.70$ and $pK_2 = 9.85$. Crosses indicate observed values for pH as given in Tables I and II. Circles indicate results obtained in other titrations (concentration 0.01). Triangles give results with equimolar mixtures of the hydrochloride with NaOH and HCl respectively in k_1 and k_2 curves.

pH of solutions of the bihydrochloride. The following are glass electrode determinations for different concentrations of the disalt.

Concentration	pH Q ₂ HCl Observed	pH HCl Observed	pH Q ₂ HCl Calculated
0.1	2.51		2.58
0.01	3.10	2.20	3.08
0.001	3.66	3.12	3.58
0.0001	4.32	4.11	4.08

The very powerful acid hydrolysis of the salt will be evident from the pH values for HCl at the same concentration. In column 4 are given the calculated values of the pH by equation (11), pK_2 being taken as 9.85.

SOLUBILITY

On addition of NaOH to a solution of a quinine salt, the alkaloid separates out immediately its solubility is exceeded as an apparently amorphous turbidity. In this form of precipitation the solubility is but little affected by temperature.

Where the solubility of the amorphous form has not been exceeded, there may still separate out a deposit of fine needle shaped crystals.* These may take minutes, hours or days to form, depending upon the concentration. The solubility of this form is considerably affected by temperature.

The following are determinations of the solubility of the amorphous form, the concentrations noted being those giving the first trace of opalescence. Mixtures for the first two columns were made to contain equivalent amounts of hydrochloride and NaOH. In the last two columns the amount of the hydrochloride was arranged to give an additional concentration of the salt as noted.

Temperature °C.	Solubility in			
	Water	0.9 per cent. NaCl	0.005 QHCl	0.05 QHCl
35	0.0017	0.0015	0.002	0.0036
30	0.0016	0.0015	0.0018	
25	0.0016			
20	0.0015	0.0015	0.0018	0.0036
10	0.0015		0.0018	
5	0.00145	0.0015	0.0018	0.0036

The saturation point for the crystalline form under similar conditions is given below, the concentrations noted giving \pm readings after addition of a small pledget of crystals, as described under materials and technique. Concentrations of 0.001 and over tend to deposit spontaneously; below this concentration, deposition usually starts only after addition of crystals.

Temperature °C.	Solubility in			
	Water	0.9 per cent. NaCl	0.005 QHCl	0.05 QHCl
35	0.00115		0.0016	0.0019
30	0.00095			0.0016
25	0.0008			
20	0.0007	0.0006		0.0014
5	0.00055	0.0005		
0	0.0005	0.00045		0.0012

*With 3(H₂O) of crystallization (see Berthelot and Gaudechon, 1903).

A graph for the solubilities of the two forms is given in fig. 4.

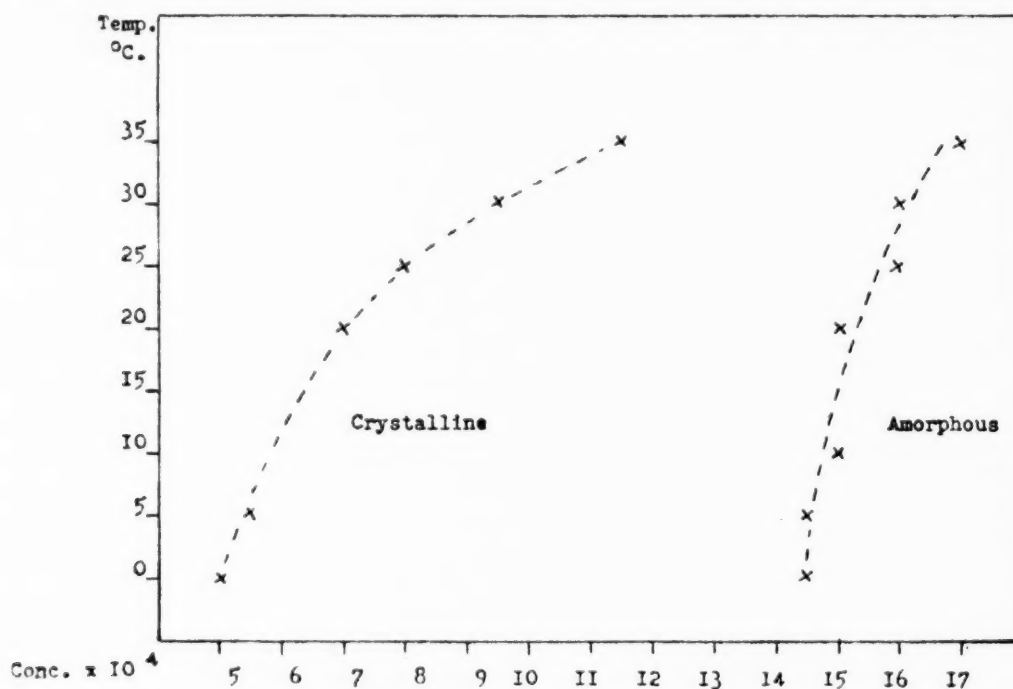


FIG. 4

Showing solubility at different temperatures of the amorphous and crystalline form of precipitated quinine alkaloid. The crosses indicate first appearance of turbidity in the amorphous form and + readings with the crystalline form.

Manz (quoted by Kolthoff, 1925) found the solubility of a saturated quinine solution at 18° C. to be 0.0006, and for quinine with three molecules of water of crystallization 0.000655. These figures are near those of the crystalline form found by me.

SOLUBILITY PRODUCT

Table III gives data obtained in this connection. The concentrations are those at which the alkaloid is just precipitated as an opalescence. Calculations

TABLE III

Solubility product for quinine

[illegible]

for both sides of the equation (equation 13) are given. For concentrations of 0.01–0.02 the mean value for the product is $10^{-8.33}$. This is very near Kolthoff's value of $10^{-8.36}$ with a salt concentration of 0.035. The value appears to increase with increasing concentration of the salt.

The crystalline form naturally gives a lower constant, viz., 1.30×7.25 or 2.80×5.70 , mean $10^{-8.52}$.

SUMMARY

1. Quinine has two constants connected with the two N atoms in the molecule. These have been determined electrometrically by the glass electrode at 20° C. as $k_1 = 5.70$ and $k_2 = 9.85$. According to Fischl and Schlossberger (1933), these constants are connected respectively with the quinuclidine and quinoline portions of the molecule.

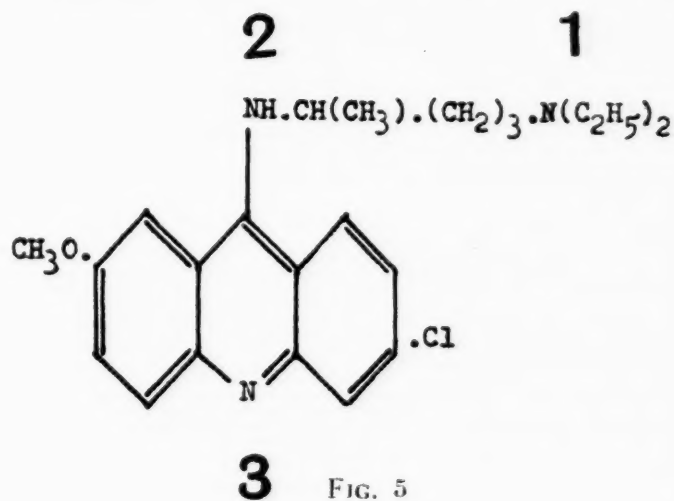
The results are very close to those of Kolthoff (1925), viz., $k_1 = 5.97$ at 16°, and $k_2 = 9.70$ at 15° C.

2. When the solubility of the alkaloid is exceeded, precipitation occurs in two forms, the amorphous and the crystalline. The former is immediate and is little affected by temperature. The solubility as determined by equivalent mixtures of QHCL and NaOH is 0.0015 at 20° C. In the presence of the hydrochloride, the solubility is 0.0019 in 0.01, 0.0027 in 0.02, and 0.0036 in 0.05 molecular concentration. The solubility product for usual concentrations of the salt (0.01–0.02) is $10^{-8.33}$.

Solubility of the crystalline form is considerably affected by temperature. At 20° C. in the presence of crystals the solubility is 0.0007. A temperature solubility curve is given.

II.—ATEBRIN CONSTITUTION

The constitutional formula for atebtrin, as given by Mauss and Mietzsch (1933), is as follows (see also Schulemann, 1935; Mietzsch and Mauss, 1934).



There are three N atoms at (1), (2) and (3) respectively, each of which might conceivably give rise to a dissociation constant.

According to Schmidt (1932), acridine is a weaker base than quinoline, and it might therefore be expected that dissociation from the N atom at (3) in atebirin will be weaker than that from the second constant of quinine, viz., 10^{-10} approximately. Since the two ascertained constants in atebirin have been found to be of the order of 10^{-4} and 10^{-6} , it is unlikely that either of these is due to the acridine ring. If, as seems possible, there is a third constant relating to (3), this must be too weak to be readily determined. Of the two constants determined, one must be assumed to be at (1) and the other at (2), but which N atom is concerned with which constant must be left for the present undecided.

MATERIALS AND TECHNIQUE

Both the hydrochloride and the base used were kindly furnished to me by Bayer and Co. The monosalt, as I was informed by Professor Schulemann and Dr. Mietzsch, cannot be prepared in the solid form, as they have observed its partition under circumstances where it should be formed into equivalent parts of base and the bihydrochloride.

If atebirin base be rubbed up with one equivalent of HCl, or if a solution of the disalt be half neutralized with NaOH, there results a mixture of disalt and precipitated base. But under such conditions the development of an orange coloration may be noted. In titrations also where the monosalt should be present in any amount, the orange coloration is very noticeable. Further, if such a solution be evaporated to dryness, there is deposited, along with the typical yellow disalt, a characteristic bright red solid, which would appear to be the monosalt.

The reason why the monosalt cannot be prepared in the pure form would seem to be due to two causes, viz., proximity of the two constants, and low solubility of the base. Since proximity of the constants is of the order of $10^{2.5}$,* a considerable concentration of undissociated base begins to be present as titration of the disalt towards the monosalt proceeds, and, since this concentration of base cannot pass beyond that imposed by its low solubility, the reaction after reaching this point continues only as precipitation of base, no more monosalt being formed.

*Note.—The unusual position occupied by atebirin in this respect is well shown by the fact that, in the long list of natural alkaloids determined by Kolthoff, only one, viz., emetin, has two constants nearer than 10^4 , whilst in this case the solubility of the base is much greater (0.002 as against 0.00008 for atebirin). It is possible, however, that, in the class of synthetic substances to be dealt with, similar or even greater insolubility and nearness of constants may exist.

Owing to these conditions, titration alone does not enable one to determine the constants, and additional methods are necessary. The methods used have been an extraction method following the lines of that employed by McCoy (1908) and Chandler (1908) for dibasic acids, and a solubility method which promises to be of considerable utility in this type of substance.

For most purposes the dihydrochloride was used. As the pH of samples of the salt was somewhat variable (probably due to the presence of traces of mono-salt), it was for titration purposes recrystallized from alcohol. So prepared, the salt gives in 0.02 solution a pH of approximately 4.7, which is the turning-point as determined by titration. The dihydrochloride is given by Mauss and Mietzsch as crystallizing with 2 molecules of water with a molecular weight of 508.5. As in the present case the salt was precipitated from alcohol, it was taken as free from water with $mw = 472.5$.

For the determination of concentrations of atebtrin, where these were not known, colorimetric methods have been used against known dilution standards, the comparison being made after conversion of all atebtrin present to the disalt.

For brevity, the dihydrochloride is referred to in the text and Tables as $A_2(HCl)$.

DETERMINATION OF THE CONSTANTS BY TITRATION

First dissociation constant

Titration of the disalt into the range of the first constant can only be carried out in great dilution, owing to precipitation of the base. Numerous titrations, however, have been made of saturated solutions of the base prepared by shaking with ether and water. Such solutions give a somewhat variable pH of 9.4–9.7, the concentration of total base being of the order 8×10^{-5} . Addition of very small increments of acid at once brings about a swing in the pH to about 8.7, followed more or less normally by the curve for k_2 . The explanation appears to be that in such high dilution the base is already largely dissociated, so that only the extreme end of the dissociation curve for k_1 is being explored. The readings are of no value in ascertaining the constant, but they show that even in very dilute solution the base gives a high pH.

Second dissociation constant

Titration. With moderately dilute solutions, the disalt can be titrated by addition of NaOH through a considerable part of the curve for k_2 . It is necessary, however, to take note of the interference from k_1 for values of $a < 0.60$. In Table IV are given the data obtained from mixtures of the disalt and NaOH.

Correction for $[B]$ has been made by the use of equation (9), giving to k_1 the value 3.88, as elsewhere determined.

At half neutralization, a value of 6.47 is given, the mean for $\alpha = 0.40 - 0.60$ being 6.48. This value is somewhat higher than that given by the ether method (6.37) and by hydrolysis of the salt (6.35), but is very near that given by the solubility method (6.46). The curve obtained approximates to the theoretical curve, but shows departure at low and noticeably also at high values of α . This latter appears not to be accidental, as it has occurred consistently in numerous titrations. As the results from titration at this level appear likely to be more reliable than those obtained by more indirect methods, the pk_1 value has been taken as 6.47.

TABLE IV

Showing determination of pk_2 for atebirin by titration of 1.6 c.cm. 0.02 A2(HCl) with NaOH in total volume of 100 c.cm. at 20° C.

Concentration A2(HCl) M $\times 10^6$	Concentration NaOH N $\times 10^6$	$[B]$ $\times 10^6$	$[OH^-]$ $\times 10^6$	$[B^-]$ $\times 10^6$	$[B^{++}]$ $\times 10^6$	α	$\log \frac{[B^{++}]}{[B^-]}$	pH	pOH	pk_2
320	0							5.42	8.64	
"	20			20.00	300.00	0.94	1.18	6.18	7.88	6.70
"	40			40.00	280.00	0.87	0.85	6.60	7.46	6.61
"	60			60.00	260.00	0.81	0.64	6.81	7.25	6.61
"	80			80.00	240.00	0.75	0.48	6.98	7.08	6.60
"	120	0.15	0.16	119.54	200.31	0.63	0.23	7.27	6.79	6.56
"	160	0.41	0.34	158.84	160.75	0.50	0.00	7.59	6.47	6.47
"	200	0.92	0.62	197.54	121.54	0.38	-0.21	7.85	6.21	6.42
"	240	1.78	1.00	235.44	82.78	0.26	-0.46	8.06	6.00	6.46
"	280	3.73	1.78	270.76	45.59	0.15	-0.77	8.31	5.75	6.52
"	320	9.80	4.36	297.64	14.16	0.046	-0.32	8.70	5.36	6.68
Mean									0.40-0.60	6.48

pH of solutions of the disalt and equivalence point. A 0.02 solution of the disalt prepared by crystallization from alcohol gave for 5 different preparations a mean value of pH 4.67. Weaker concentrations give higher values.

Determinations of the point of maximum pH change when mixtures of the monosalt and disalt are titrated to disalt are given below. It is evident that the point varies with the total concentration, i.e., the concentration of disalt when all salt present is in this form.

$[B^+] \times 10^5$	$[B^{++}] \times 10^5$	Total atebtrin $\times 10^5$	pH original fluid	Equivalence point	pk_1
	2,000	2,000	4.67	4.67	6.36
97	213	310	7.51	5.10	6.32
101	81	180	7.82	5.14	6.46
92	53	145	8.03	5.26	6.32
78	27	105	8.20	5.31	6.36
21	3	24	9.06	5.66	6.30
Mean					6.35

In the last column are given values of pk_2 calculated from the turning point by means of equation (12). The mean pk value arrived at is 6.35.

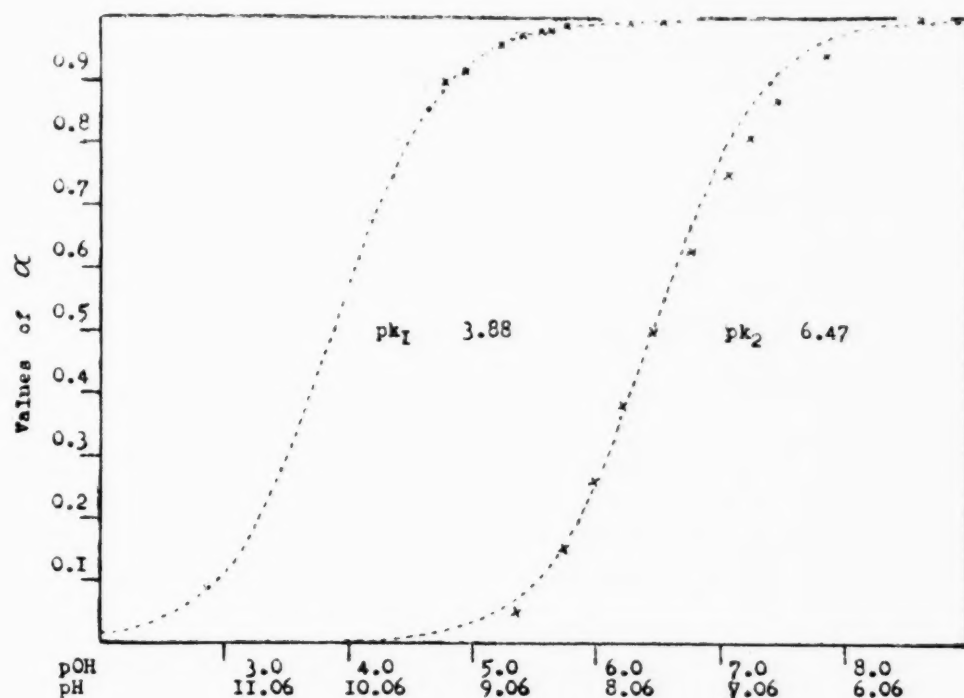


FIG. 6

Showing dissociation curves for atebtrin. The dotted lines give the theoretical curves for $pk_1 = 3.88$ and $pk_2 = 6.47$. The crosses indicate observed values for pH as given in Tables IV and V.

Third dissociation constant

The structure of atebtrin suggests the possibility of a third constant. This is rather supported by the fact that the disalt can very readily take up acid, which it does not part with easily. This is especially noticeable when precipitating the bihydrochloride from a solution of the base in ether with dry HCl. If an excess of acid be used, the product after many washings and recrystallization from alcohol may still give a pH in 0.02 solution as low as 2.5.

No third constant has, however, been demonstrated on titration of the disalt with HCl to a pH of 3.52, as is shown by the following data, where 1.6 c.cm. of 0.02 disalt has been titrated with HCl in a total volume of 100 c.cm.

c.cm. 0.02 HCl	Concentration added HCl	pH of mixture	pH of HCl only
0.1	0.00002	4.72	4.70
0.2	0.00004	4.44	4.40
0.4	0.00008	4.12	4.10
0.6	0.00012	3.95	3.92
0.8	0.00016	3.85	3.80
1.0	0.00020	3.64	3.62
1.2	0.00024	3.64	3.62
1.4	0.00028	3.57	3.55
1.6	0.00032	3.52	3.49

A third constant, if present, must therefore be weaker than $k_3 = 10^{-11}$.

DETERMINATION OF THE CONSTANTS BY THE ETHER-EXTRACTION METHOD

Partition coefficient. Attempts to arrive at satisfactory results through shaking solutions of base in ether and water, or alternatively by shaking mixtures of the disalt and NaOH with ether, gave very much higher coefficients than are believed to be the true values, and also no constant coefficient. The assumption that the dissociated base in such solutions was equivalent to $[\text{OH}^-]$ also yielded no constant coefficient (see footnote in section on formulation).

Since the object was to obtain the coefficient for the molecular substance, undissociated base B, it was then thought that the most direct way to attain this object was to partition under conditions where ionization was completely suppressed, and hence where only the undissociated form of the base would be present. Experiments had shown that, after addition of a double equivalent of NaOH to the disalt, further addition led to a rapid reduction in the solubility of total base, but that, after a certain concentration of NaOH had been reached, a relatively constant solubility resulted. Such solubility was taken to be that of the undissociated base. Determinations of partitioning made on this plan gave reasonably concordant results, as shown below.

c.cm. A2(HCl) added	Concentration of atebirin in aqueous layer $\times 10^5$	Concentration of atebirin in ether layer $\times 10^5$	Coefficient
5	0.825	1,820	0.00045
10	1.300	3,640	0.00036
15	1.875	5,460	0.00034
20	2.500	7,280	0.00034
25	2.700	9,100	0.00030
30	3.000	10,920	0.00027
Mean			0.00034

The determinations were made by shaking 70 c.cm. of aqueous mixture and 12 c.cm. of ether, giving a final volume after shaking of 76 c.cm. aqueous and 5.5 c.cm. ether. The mixtures were made by adding various amounts of the disalt as shown and NaOH to give a final concentration of 0.04 mol. The concentrations of atebirin in the ether and aqueous layers after shaking were then determined colorimetrically as disalt. The determinations were made at 20° C.

A number of precautions were found to be of vital importance. It is essential that the ether used be free from all traces of acid or alcohol, and that used was redistilled over sodium after treatment with strong NaOH. It is essential that the temperature should remain strictly unchanged during settling or any subsequent manipulation. Otherwise there is likely to be serious error (a) owing to increased solubility of the ether at a lower temperature, leading to solution of minute (submicroscopic) droplets of ether and the yielding up by them to the aqueous layer of their considerable content of base; (b) an opposite effect from raising of the temperature, causing formation of droplets and the taking up by these of base. Very small changes, e.g., a degree, are sufficient entirely to vitiate results under certain circumstances. The haze produced merely by momentarily handling a tube of saturated ether in water will serve to demonstrate the readiness with which such phenomena occur.

Determination of k_1 and k_2 . In Table V are given the data relative to a number of determinations of the constants by this method. The proportions of ether and aqueous layers were as already noted, the latter being made to contain various proportions of the disalt and NaOH as noted in the Table. After shaking, 50 c.cm. of the aqueous layer was removed and titrated with 0.02 or 0.002 HCl, thus giving the total titratable base in terms of monovalent base (= C). The initial reading before addition of acid gave also the pH.

In columns 1 and 2 of the Table are given M and N, i.e., if 10 c.cm. of 0.02 A2(HCl) was present in the original 70 c.cm., the concentration M after shaking is $10/76 \times 0.02 = 0.002632$. Column 3 gives C. To determine the constants it is necessary to know $[B]$, $[B^+]$, $[B^{++}]$ and $[OH^-]$. The values are then readily calculated from equations (3) and (4).

Since $N = [NaOH]$, the concentration of undissociated base removed by the ether from the aqueous layer is $\frac{1}{2}(N - C) = E$ (column 4), and $[B]$ in the ether is $76/5.5 \times E = E'$ (column 5). $[B]$ in the aqueous is therefore $E'p$ where p is the coefficient, i.e., $[B] = E'p$.

Again, since the total concentration of titratable base in monovalent terms is clearly equivalent to N as previously used, $[B^+] = C - 2[B] - [OH^-]$. $[B^{++}]$ is as in equation (8), except that an amount E removed by the ether must be allowed for, i.e., $[B^{++}] = M - E - [B] - [B^+]$. $[OH^-]$ is given by the pH ($pOH = pk_w - pH$).

The constants as given by the means in the Table are $k_1 = 3.89$ and $k_2 = 6.37$, the pk difference being 2.49.

TABLE V
Showing determination of the constants by the ether method. p taken as 0.00034

Concen- tration $A_2(HCl)$ $M \times 10^6$	Concen- tration $NaOH$ $N \times 10^6$	Concen- tration titratable base $C \times 10^6$	$[B]$ removed by ether $E \times 10^6$	Concen- tration $[B]$ in ether $E' \times 10^6$	$[OH^-]$ $\times 10^6$	$[B]$ $E'p \times 10^6$	$[B^+]$ $\times 10^6$	$[B^{++}]$ $\times 10^6$	$\log \frac{[B^+]}{[B]}$	$\log \frac{[B^{++}]}{[B^+]}$	pOH	pk ₁	pk ₂
2632	4605	472	2,067	0.0285	2.20	9.69	450	105	1.67	-0.63	6.29	3.99	6.29
"	3947	796	1,576	0.0218	1.38	7.41	780	269	2.02	-0.46	6.32	3.84	6.32
"	3289	936	1,176	0.0162	0.93	5.51	924	526	2.23	-0.24	6.27	3.80	6.27
"	2632	1,024	804	0.0111	0.58	3.77	1,016	808	2.40	-0.10	6.34	3.84	6.34
"	1974	972	501	0.0069	0.28	2.35	967	1,162	2.61	0.08	6.47	3.94	6.47
"	1316	840	238	0.0033	0.19	1.12	838	1,555	2.87	0.27	6.51	3.93	6.51
Mean												3.89	6.37

DETERMINATION OF THE CONSTANTS BY THE SOLUBILITY METHOD

The solubility of B is determined later as 0.000016 (see under solubility). In any mixture, therefore, in which the base is just precipitated, the concentration of B is known, and calculations as for the ether method can be made. Data obtained in this respect are given in Table VI. The determinations were made with CO₂-free water in 100 c.cm. flasks kept corked and nearly filled with the fluid, avoiding exposure in the manipulations as much as possible. Illumination was arranged to minimize fluorescence whilst showing up turbidity.

Excluding outlying values, indicated by asterisk, the constants as shown by the means are $k_1 = 3.87$ and $k_2 = 6.46$. The first is practically the figure found with the ether method, and the second the value for k_2 given by titration, though such a correspondence is to some extent probably accidental. There is clearly a change in the values with the higher salt concentrations, whether from increased solubility of B, change in p_k , or activity of the salts is not known. Whilst the method might be expected to be rather approximate, it seems to have given good determinations, and it has the advantage of being most applicable in just those cases where high insolubility of the base might make determinations difficult by other methods.

SOLUBILITY

On the addition of NaOH to a solution of the disalt, the base is precipitated as an opalescence or turbidity. Separation as flakes is much less marked than with quinine, and the turbidity is very readily redissolved. Hence, solubility determinations can be made more readily and accurately than with quinine. A fine opalescence is, however, very readily dissolved by CO₂ from the air, if precautions are not taken. So far, no crystalline form of deposit has been seen in the mixtures, as with quinine, and all data relate to amorphous deposit. Crystals of base may, however, be formed from aqueous solution under certain circumstances, and are readily deposited from ether and alcohol. Solubility of the base is very little affected by temperature from 0–35° C.

Table VII shows determinations of solubility of the base in water and in varying concentrations of NaOH. The concentrations noted under solubility are those at which the first sign of opalescence appears. For such determinations, an illumination should be chosen to reduce as much as possible the fluorescence. The results indicate the following solubilities of atebirin base: in water (diss. + undiss.) 8.4×10^{-5} ; in water (undiss.) 1.6×10^{-5} ; in water saturated with ether (undiss.) 4×10^{-5} .

TABLE VI
Showing determination of constants by solubility method

c.cm. 0.02 A ₂ (HCl) to 100c.cm. total	c.cm. 0.02 NaOH to 100c.cm. total	Concen- tration A ₂ (HCl) M × 10 ⁶	Concen- tration NaOH N × 10 ⁶	[OH ⁻] × 10 ⁶	[B] × 10 ⁶	[B ⁺] × 10 ⁶	[B ⁺⁺] × 10 ⁶	$\log \frac{[B^+]}{[B]}$	$\log \frac{[B^{++}]}{[B^-]}$	pOH	pk ₁	pk ₂
0.80	0.95	160	190	16.0	16	142	2	0.95		4.79	3.84	
1.0	1.10	200	220	10.7	"	177	7	1.05		4.97	3.92	
2.0	2.05	400	410	4.6	"	373	11	1.37		5.34	3.97	
3.0	3.00	600	600	3.9	"	564	20	1.55	-1.45	5.41	3.86	6.86*
4.0	3.84	800	768	2.8	"	733	51	1.66	-1.16	5.56	3.90	6.72*
5.0	4.50	1,000	900	2.5	"	866	118	1.73	-0.87	5.61	3.88	6.48
10.0	8.30	2,000	1,660	1.7	"	1,626	358	2.01	-0.66	5.76	3.75	6.42
20.0	15.85	4,000	3,170	1.2	"	3,137	847	2.29	-0.57	5.91	3.62*	6.48
Mean											3.87	6.46

*Omitted from mean.

TABLE VII

c.cm. 0.02 A2(HCl) in 100 c.cm. total	c.cm. 0.02 NaOH in 100 c.cm. total	Concen- tration NaOH	Solubility	pOH	Remarks
0.42	0.84	water	0.000084	4.36	Undissociated and dissociated
0.20	1.40	0.0002	0.000040	3.70	
0.12	2.24	0.0004	0.000024	3.40	
0.10	5.20	0.001	0.000020	3.00	
0.09		0.004	0.000018	2.40	
0.08		0.02	0.000016	1.70	Undissociated only
0.08		0.05	0.000016	1.30	
0.08		0.1	0.000016	1.00	
0.20		0.05*	0.000040		Solubility undissociated base in water saturated with ether

*Saturated with ether.

SOLUBILITY PRODUCT

If the solubility of B in water be taken as 0.000016, the value of $k_1 \times [B]_{\text{sat.}}$
 $= 10^{-3.88} \times 10^{-4.8} = 10^{-8.68}$.

For the left-hand side of the equation are data as given in Table VI, each entry in which may be regarded as an experiment designed to give this value. The results are summarized in Table VIII.

TABLE VIII

Total A	$[B^+] \times 10^6$	$[OH^-] \times 10^6$	$[B^+] \times [OH^-] \times 10^{12}$	L
0.00016	142	16.0	2272	$10^{-8.64}$
0.0002	177	10.7	1894	$10^{-8.77}$
0.0004	373	4.6	1716	$10^{-8.77}$
0.0006	564	3.9	2200	$10^{-8.68}$
0.0008	733	2.8	2052	$10^{-8.89}$
0.001	866	2.5	2165	$10^{-8.66}$
0.002	1,626	1.7	2764*	
0.004	3,137	1.2	3764*	
Mean			2050	$10^{-8.69}$

*Omitted from mean.

The value obtained in the Table is practically identical with that given for the product of k_1 and $[B]_{\text{sat.}}$ and the solubility product for atebrin has been taken as $10^{-8.68}$.

SUMMARY

1. Atebrin possesses two dissociation constants, probably both connected with the amine portion of the molecule, one very strong, $\text{pk}_1 = 3.88$, the other weaker, $\text{pk}_2 = 6.47$. There are some indications of a third constant, but if such exists it is weaker than $\text{pk}_3 = 11.0$. If a third constant exists, it is probably related to the acridine ring.

2. The base is soluble in water to the extent of $10^{-4.08}$ (0.000084); the undissociated base to the extent of $10^{-4.8}$ (0.000016). The solubility product is $10^{-8.68}$.

3. Owing to the comparative nearness of the constants ($\text{pk}_2 - \text{pk}_1 = 2.49$) and to the very low solubility of the undissociated base, the monosalt (corresponding to the hydrochloride of quinine) cannot be readily prepared in an isolated form. Such a salt, however, exists, and can be demonstrated in solid form, as well as by colour in solutions. The solid salt is a bright red.

4. A new solubility method of determining pk values is described, likely to be useful in the case of substances where the base is highly insoluble.

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STUDIES ON THE EFFECT OF VARIOUS FACTORS ON THE INFECTION RATE OF *ANOPHELES ELUTUS* WITH DIFFERENT SPECIES OF *PLASMODIUM*

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The main object of the experiments reported below was to ascertain the factors concerned in the infectivity of the various species of *Plasmodium* for *A. elutus*. Darling (1912), in his classic studies, pointed out that, while a minimum of 12 gametes per c.mm. of blood was necessary for infecting *Anopheles*, it must be understood that several factors are concerned in infection, such as the proportion of male to female gametes, number and activity of leucocytes, and immunity of mosquitoes, racial and individual. He cites experiments in which the gametes exflagellated *in vitro* and yet failed to infect.

The complexity of the problem, and its importance in relation to the epidemiology of malaria, seemed to us to warrant further study of this question. Moreover, it is becoming more and more apparent that the biology of the adult *Anopheles* varies with season and is greatly influenced by the conditions under which they breed; it seemed important, therefore, that experimental infections be carried out with mosquitoes bred under conditions as nearly as possible simulating those prevailing in nature. Insects bred in the laboratory under constant temperatures and infected under standardized conditions cannot be expected to yield results corresponding to those obtained with *Anopheles* breeding under natural conditions. In all our experiments we have, therefore, used mosquitoes bred in containers, kept out-of-doors, and subject to the variations in temperature, food, etc., characteristic of the period of the year when the experiments were carried out.

The results presented below constitute a summary of our observations during a period of two and a half years. Over 200 infection experiments have been carried out; for various reasons, however, a considerable number of the experiments had to be discarded, and only those are included in which the observations were complete, and where the certainty existed that only one species of *Plasmodium* was involved.

1. Relation of Temperature to Exflagellation

Since exflagellation is the first prerequisite to infection, we studied the presence and rate of exflagellation of the three species of *Plasmodium* at different temperatures :—

In *P. vivax* exflagellation occurred promptly at moderate temperatures. At the relatively low temperature of 15–16° C. exflagellation began after 20 minutes, but was not complete in 2 hours. At 22° C., as well as at 27° C., exflagellation started in 5–10 minutes and was completed in 10–15 minutes. The highest temperature at which exflagellation was observed was 29° C.

In *P. falciparum* exflagellation was observed at a minimum temperature of 14° C., and at a maximum of 33° C. However, the process is at all temperatures slower than in the case of *P. vivax*. The rate at different temperatures was as follows :—

at 14° C.	began after 1 hour and 55 mins.
„ 15° C.	„ „ 1 „ „ 45 „
„ 16° C.	„ „ 1 „ „ 45 „
„ 21·5° C.	„ „ „ „ 20 „
„ 33° C.	„ „ „ „ 10 „

In *P. malariae* exflagellation was noted at 22° C. and at 27° C. The process is exceedingly slow : at 21–22° C. it began only after 30 minutes, while at 27° C. it began after 10 minutes.

In so far as our observations go, all three species exflagellate readily at temperatures of 21° C. and over. The rate of exflagellation varies, however : it is most rapid in *P. vivax* and least so in *P. malariae*. At a temperature of 15–16° C. the process is greatly retarded in all species, and the individual differences are more striking.

2. Relation of Number of Gametes to Infection

If the experiments with *P. falciparum* are arranged in the order of increasing number of gametes per c.mm. of blood, it appears that fewer failures to infect were obtained when the number of gametes was relatively high. There is, however, no uniform correlation, and the results are highly irregular. At times, only 5 gametes per c.mm. of blood, or only 1–2 per thick drop, may give a relatively high percentage of infected mosquitoes, while large numbers of gametes may give negative results. The data are summarized in Table I.

The first three columns in the Table give the observations of all experiments, and the last three only of those experiments in which exflagellation was actually observed. The last column gives the ratio of positive to total number of experiments in the exflagellated group. It will be noted that there were considerable

TABLE I

Relation of number of M.T. gametes to infection of *Anopheles* (*A. elutus*)

No. of gametes per c.mm.	All experiments			Experiments in which exflagellation was observed			
	No. of <i>Anopheles</i> dissected	No. of <i>Anopheles</i> positive	Percentage of <i>Anopheles</i> infected	No. of <i>Anopheles</i> dissected	No. of <i>Anopheles</i> positive	Percentage of <i>Anopheles</i> infected	Ratio of + experiments to total number
5-10	28	5	18.0	5	1	20.0	1 : 1
20	28	3	10.7	6	0	0.0	0 : 1
30-40	41	7	17.0	33	7	21.2	2 : 3
45-50	80	12	15.0	35	11	31.4	2 : 2
75-95	63	0	0.0	52	0	0.0	0 : 3
110-120	28	23	82.1	28	23	82.1	3 : 3
140-190	43	3	7.0	10	2	20.0	1 : 1
200	45	11	24.9	45	11	24.9	4 : 4
210-240	51	20	39.2	50	19	38.0	3 : 5
270-300	58	17	30.0	33	17	51.5	3 : 3
340-1,000	51	11	21.6	27	6	22.2	3 : 4
1,000+	69	17	24.6	69	17	24.6	4 : 5

variations in every group of experiments, that even in those experiments in which exflagellation was recorded the percentage of mosquitoes infected by cases with 5-40 gametes was the same as with 140-200 or 340-1,000, and that the best results were obtained when the number of gametes was 110-120. Likewise, there were negative experiments with cases having a large number of gametes, as there were with cases with small numbers. It should be noted that in this Table we have excluded experiments carried out at low temperatures, as well as those with carriers who were under treatment.

In the case of *P. vivax*, only 2 of 13 experiments were negative. In one of these, only 3 mosquitoes were dissected, and in the other there were 12, all of which were negative. In all these cases the number of gametes was small, and it was difficult to establish the exact number.

In *P. malariae* the results were generally the same as with *P. falciparum*. There were 9 positive experiments and 25 negative ones. Of the positive experiments, one was with a case which had 18 gametes per c.mm. ; 3, 30-50 gametes ; 3, 70-75 gametes ; one case had 120 ; and one 164 gametes. At the same time, there were 15 negative experiments, with cases having 25-60 gametes. The results are given in Table II.

TABLE II

Quartan experiment : relation of number of gametes to infection

No. of the experiment	Exflagellation	No. of gametes per c.mm.	Positive experiment		Negative experiment
			No. of mosquitoes dissected	No. found infected	No. of mosquitoes dissected
86	—	3			15
88	—	5			13
90	pres.	10			10
82	—	14			8
94	—	17			12
87	—	17			21
93	none	18			23
97	pres.	18	17	1	
89	—	22			10
80	—	23			8
103	—	24			10
85	pres.	25			10
109	—	25			17
84	—	26			8
108	—	26			9
81	pres.	30	11	2	
83	—	31			16
102	—	30			10
98	pres.	33			18
96	—	34			15
92	pres.	36			26
111	none	40			14
95	pres.	41	10	5	
79	none	45			9
101	—	49	5	1	
105	pres.	60			8
107	—	60			7
110	—	70	10	4	
91	pres.	72	11	7	
104	—	75	7	2	
99	pres.	120	16	8	
100	pres.	164	2	1	
106	pres.	360			9

— = not examined.

It seems clear, therefore, that in tropical and quartan malaria an infective carrier is not necessarily a person having a large number of gametes in his circulation. Other factors are obviously involved.

3. *Effect of Ratio of Micro- to Macrogametes*

Exflagellation shows the condition of the microgametocytes, but the state of the macrogametocytes remains obscure. If we group our experiments with M.T. carriers according to the ratio of the two types of gametes, an interesting point appears. Of 12 carriers in whom the ratio was 2 or 3 : 1, 5, or 41·7 per cent., gave positive infections. On the other hand, of 19 carriers in whom the ratio was about 1 : 1—that is, equal numbers of the two types of gametes—the number of positive experiments was 13, or 68·4 per cent. It is reasonable to suppose that a disproportion in the two types of gametes indicates a state of abnormality in the gametes, leading to fewer infections, as well as to infections of lesser intensity. However, although this observation throws some light on the complexity of the problem, it still remains to account for the negative results in cases with equal numbers of gametes of the two sexes.

4. *Relation of Season*

As stated above, the mosquitoes used in these experiments were bred under natural conditions, and were consequently subject to climatic influences affecting mosquitoes bred in the marsh. We therefore regrouped our data on the basis of season. It seemed likely that, in view of the different habits manifested by these insects at different periods of the year, they might also show differences in their susceptibility to infection. The results are shown in Table III.

TABLE III

Positive and negative M.T. infection experiments at different months (all experiments)

	No. of experiments	No. positive	Percentage positive
May	3	0	0·0
June	8	6	75·0
July	8	5	67·5
August	9	7	77·7
September ...	12	8	66·7
October	13	11	84·6
November ...	4	1	25·0
Total	57	38	66·7

It appears from Table III that the percentage of positive experiments was practically the same during the months June to October, but was strikingly lower in May and November.

It happens, however, that the number of experiments both in May and November is much less than in other months, and one may, therefore, properly question the significance of these figures. However, if the results are retabulated

on the basis of the number and percentage of mosquitoes infected, much more striking differences appear.

TABLE IV

Monthly incidence of infected mosquitoes (*P. falciparum*) in experimental infection experiments (experiments in which exflagellation was negative are excluded)

	May	June	July	Aug.	Sept.	Oct.	Nov.
No. of experiments ...	3	7	5	8	12	12	3
No. positive ...	0	6	4	8	8	11	1
Percentage positive ...	0	85.7	80	100	66.7	91.7	30.3
No. of mosquitoes dissected	52	89	31	92	99	143	28
No. positive ...	0	46	14	18	18	48	4
Percentage positive ...	0	51.6	45.0	19.5	18.0	33.6	14.3

These data are shown in Table IV. In this Table all experiments are excluded in which no exflagellation occurred. The data presented in this Table bring out two points:—The number of positive experiments is more or less the same during all months except May and November. The percentage of mosquitoes infected varies, however, considerably from month to month: it is highest in June and July, drops in August and September, rises again in October, and falls in November. Although the numbers of mosquitoes involved are relatively small, the results are highly suggestive, and possibly also significant.

Experiments with quartan parasites are more difficult to evaluate, because the number of positive experiments is relatively small, and the intensity of infection in the positive experiments is low. The results are given in Table V.

TABLE V

Incidence of infections with quartan parasites at different months

	May	June	July	Aug.	Sept.	Oct.	Nov.
No. of experiments ...	6	8	1	5	3	7	1
No. positive ...	0	4	1	2	1	1	0
Percentage positive*	0	50.0	—	40.0	33.3	14.3	—
No. of mosquitoes dissected	88	115	2	44	23	90	8
No. positive ...	0	18	1	3	2	7	0
Percentage positive ...	0.0	15.7	—	7.0	8.7	7.8	0.0

* Where only one experiment was made, the percentage is not given, because it would be misleading.

In general, there seems a certain correspondence with the results obtained with M.T. parasites. Negative results were obtained in May, and the highest positive results were in June. In August, September and October, 7–8 per cent.

of the mosquitoes were infected, while in June the percentage was 15·7, or twice as high.

The infections with *P. vivax* differ from those with the other two parasites. The number of experiments is small and does not cover the entire year. But even these few experiments bring out certain marked differences. Contrary to the two other parasites, this one gives maximal infections during May and poorer results in June. Whether these results are accidental or not still remains to be determined. The data are summarized in Table VI.

TABLE VI
Incidence of infection with *P. vivax* at different months of the year

	May	June	Aug.	Sept.	Oct.	Nov.
No. of experiments	3	4	1	1	3	2
No. positive	3	3	1	1	3	1
Percentage positive	100	75	—	—	100	50
No. of mosquitoes dissected ...	25	38	4	6	25	16
No. positive	25	11	4	6	17	3
Percentage positive	100	29·0	—	—	68·0	18·7

It is of interest to compare the infectivity of the different species of *Plasmodium* for the same species of *Anopheles*. The differences are striking, and are certainly of significance in relation to the epidemiology of the disease and the relative incidence of the three types of malaria.

TABLE VII
Comparative infectivity of different species of *Plasmodium* for *A. elutus*

Month	<i>P. vivax</i>			<i>P. falciparum</i>			<i>P. malariae</i>		
	No. of mosquitoes		Per-centage positive	No. of mosquitoes		Per-centage positive	No. of mosquitoes		Per-centage positive
	Dissected	Positive		Dissected	Positive		Dissected	Positive	
May	25	25	100·0	52	0	0	88	0	0
June	38	11	29·0	89	46	51·6	115	18	15·7
July				31	14	45·0			
Aug.	4	4	—	92	18	19·5	44	3	7·0
Sept.	6	6	—	99	18	18·0	23	2	8·7
Oct.	25	17	68	143	48	33·6	90	7	7·8
Nov.	16	3	18·7	28	4	14·6	8	0	0
Total	114	66	5·79	534	148	27·7	370	31	8·4
				454*	144	31·8			

* This row of figures represents the results if May and November are excluded. Even during months when infection occurs readily, the *percentage* of mosquitoes infected is significantly less than with *P. vivax*.

The comparative data are shown in Table VII. Apart from the seasonal differences already referred to, it will be noted that *P. vivax* infected about 58 per cent. of the mosquitoes dissected, *P. falciparum* 27·7 per cent. (about half), and *P. malariae* only 8·4 per cent. It will be of interest to note the relation obtained in other species of *Anopheles*.

It would seem, therefore, in so far as these results warrant any deduction, that the same species of *Anopheles* has different degrees of susceptibility to different species of parasites, as well as different sensitivity to the same parasite at different periods of the year. Both the parasites and the *Anopheles* seem to be subject to the influence of external factors, and their behaviour follows a seasonal rhythm. These findings correspond, in a general way, with the results of dissections of wild mosquitoes collected in various places, and to some extent they offer the basis for an explanation of these results. Tables VIIIa and

TABLE VIIIa

Monthly incidence of infected *A. elutus* among mosquitoes caught in the highly infected Huleh region

Month	No. of mosquitoes dissected	No. positive			Percentage infected	
		Oöcysts	Sporozoites	Total	Sporozoites	Total
April ...	163	3	0	3	0	1·8
May ...	250	3	1	4	0·4	1·6
June ...	262	6	4	10	1·5	3·8
July ...	326	10	3	13	0·9	4·0
August ...	222	4	5	9	2·2	4·0
September ...	72	2	0	2	0·0	2·8
October ...	353	5	2	7	0·6	2·0
November ...	361	4	2	6	0·5	1·7
December ...	44	1	1	2	2·2	4·5*
Total ...	2,053	38	18	56	0·9	2·7

* This high figure is due to accumulation of infected hibernating mosquitoes.

VIIIb give the results of dissections in two different regions, one heavily infected, the other much less so. The monthly rhythm is clearly brought out, though it is less marked than in our infection experiments, owing probably to the mixture of parasites in natural infections.

TABLE VIIIb

Monthly incidence of infected *A. elutus* among mosquitoes in areas not heavily infected

Month	No. dissected	No. positive	Percentage positive
January	235	2	0.9
February	122	0	0.0
March	69	1	1.4*
April	231	1	0.4
May	377	0	0.0
June... ..	354	5	1.4
July	255	1	0.4
August	163	1	0.6
September	—	—	—
October	127	1	0.8
November	192	1	0.5
December	264	4	1.5*
Total	2,389	17	0.7

*Hibernating.

5. Effect of Temperature on the Rate of Development of the Different Species of Plasmodium in Mosquitoes

As in the experiments reported above, mosquitoes bred under natural conditions were infected and kept at the prevailing temperatures during the period of observation. The results, therefore, presumably correspond with those which obtain in nature. The data are summarized in Table IX.

It appears that in the case of *P. falciparum* the cysts remain intact at a temperature range of 11–19° C. ; they do not seem to degenerate, but neither do they develop. At 17–23° C. (average 19° C.) the evolution takes 35 days ; at 19–25° C. (average 21° C.), 23 days ; at 23–24° C., 18 days ; and at 25–31° C., 14–15 days. In other words, at temperatures prevailing in May and June, it takes 3½ and 2½ weeks respectively for these parasites to complete their development, while at temperatures prevailing in August and September the period is only 2 weeks. The higher temperature required for its development may account for the absence of positive M.T. infections during the month of May.

It is apparent that the B.T. parasites develop more rapidly at corresponding temperatures than do the M.T. parasites, and also that they develop at the low temperatures at which the M.T. parasites do not develop. At 15° C. these parasites develop slowly, whereas the M.T. remain stationary ; while at a mean temperature of 19° C. they require 24 days for evolution of sporozoites, as against 35 days required by *P. falciparum*.

The quartan parasites develop most slowly. At a mean temperature of 24° C., complete evolution requires at least 27 days, as against 18 days for

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It would seem, therefore, in so far as these results warrant any deduction, that the same species of *Anopheles* has different degrees of susceptibility to different species of parasites, as well as different sensitivity to the same parasite at different periods of the year. Both the parasites and the *Anopheles* seem to be subject to the influence of external factors, and their behaviour follows a seasonal rhythm. These findings correspond, in a general way, with the results of dissections of wild mosquitoes collected in various places, and to some extent they offer the basis for an explanation of these results. Tables VIIIa and

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The quartan parasites develop most slowly. At a mean temperature of 24° C., complete evolution requires at least 27 days, as against 18 days for

TABLE IX

Effect of temperature on the rate of development of various parasites in *A. elutus*

(a) <i>M.T. infections</i>					
Temperature range	Mean	No. of experiments	Days after infection	State of development	Remarks
11-19° C.	17° C.	1	22-50	Cysts 5-10 μ	Not developed
17-23° C.	19° C.	4	18	" 21 μ	Largest cyst
			25	" 30 μ	" "
			28-31	" 40-50 μ	" "
			35-38	<i>Sporozoites in glands</i>	
19-25° C.	21° C.	1	16	Cysts 32 μ	Largest cyst
			23	<i>Sporozoites in cysts</i>	
19-24° C.	22° C.	1	17	Cysts 20 μ	Largest cyst
23-25° C.	24° C.	8	8	" 10-15 μ	" "
			11-12	" 27-30 μ	" "
				" 20-39 μ	Range of sizes
			14-15	<i>Sporozoites in glands</i>	
			18-22		
24-26° C.	25° C.	1	8	Cyst 45 μ	Largest size
24-28° C.	26° C.	1	12	" 36 μ	" "
25-31° C.	27° C.	2	12	" 45 μ	" "
			15	<i>Sporozoites</i>	
25-31° C.	28° C.	1	14	"	
(b) <i>B.T. infections</i>					
11-22° C.	15° C.	2	16	Cyst 30 μ	} Development slow but definite; not carried to end
			19	" 30 μ	
			25	" 36 μ	
			37	" 36 μ	
17-26° C.	19-21° C.	4	8	Cysts 5-10 μ	Largest cysts
			9	" 10-15 μ	" "
			12	" 30 μ	" "
			15-16	" 30-40 μ	" "
			19-24	<i>Sporozoites in glands</i>	
(c) <i>Quartan infections</i>					
22-26° C.	23° C.	4	12-13	Cysts 5-10 μ	Largest cyst
			20-26	"	Large; chromatin present
			27-28	<i>Sporozoites in glands</i>	
26-28° C.	27° C.	1	17	Cysts	Medium size
			22	"	Big, with division of chromatin. Day of appearance of sporozoites not noted
			?		

M.T. parasites, while at 27° C. the time required is probably 24 days, as against 15 days for M.T. parasites.

The results indicate, therefore, that the three parasites have different inherent rates of development, and that in each of them the rate is regulated by the external temperature.

6. Effect of Treatment on Infectivity of M.T. Gametes

(a) *M.T. gametes—treated with atebirin.* Data are available in four experiments on the infectivity of gametes during treatment with atebirin:—

Experiment 88. Treated with quinine followed by atebirin. Quinine treatment started August 1st; atebirin started August 3rd; August 6th, many M.T. gametes; *A. elutus* fed. Of 10 mosquitoes, 5 were infected.

Experiment 89. Atebrin alone. Treatment started August 2nd; daily 0.2 gm. On August 7th, many gametes; *A. elutus* fed. Of 7 mosquitoes, 2 were infected.

Experiment 129. Atebrin, October 4th–9th, 0.3 gm. daily; October 9th, *A. elutus* fed. Of 8 mosquitoes, 6 were infected.

Experiment 131. October 10th, few gametes. Atebrin, October 9th and 10th, 0.3 gm.; October 11th–15th, 0.2 gm.; October 16th, many gametes; *A. elutus* fed. Of 14 mosquitoes, 6 were infected.

(b) *M.T. gametes—quinine treatment.*

Experiment 108. Few M.T. rings on September 11th; 0.25 gm. quinine formiate intravenously; September 11th–15th, 0.9 gm. quinine per os; September 15th, gametes scarce; *A. elutus* fed. Of 10 mosquitoes, none was infected.

Experiment 113. September 14th, M.T. rings numerous; 0.5 gm. quinine intramuscularly; September 14th–20th, 0.9 gm. quinine per os; September 21st, M.T. gametes numerous; *A. elutus* fed. Of 8 mosquitoes, 5 were infected.

Experiment 132. October 11th, few rings found; October 11th–14th, 0.9 gm. quinine and 0.2 methylene blue; October 15th, 0.3 gm. quinine. October 16th, many M.T. gametes; *A. elutus* fed. Of 28 mosquitoes, 3 were infected.

It has previously been established by various authors that quinine does not affect the M.T. gametes. These experiments confirm the findings with regard to quinine, and demonstrate that atebirin behaves in the same manner as quinine.

DISCUSSION

The data presented above, though necessarily limited, are highly suggestive. Infection experiments under field conditions is not a simple matter. The carrier is not ideal, the number of gametes is variable, and the infection of *Anopheles* is necessarily the result of single feeds. On the other hand, the presumption is that the results thus obtained are more likely to correspond to conditions prevailing in nature than are those obtained under standardized laboratory conditions.

Granting the validity of this assumption, the results reported in the present paper have more than a passing interest. It has generally been assumed that the incidence of infected *Anopheles* was a function of the incidence of carriers, and that the intensity of the infection in the mosquito depended chiefly on the

number of gametes present in the blood of the infecting carrier. Our results suggest that the vector is not altogether a passive agent in the process. *A. elutus* bred under temperature conditions corresponding to those in nature manifest different degrees of susceptibility to the various species of *Plasmodium* and to the same species at different seasons of the year. This latter, or seasonal, difference in susceptibility is particularly striking when we compare the relative monthly incidence of infected mosquitoes. For example, the *Anopheles* hatching in May were not infected by gametes of *P. falciparum*, despite the facts that in these experiments the number of gametes was 75 and 95 per c.mm., the relation of male to female gametes was about 1 : 1 or 2 : 1, and exflagellation took place. Not a single mosquito of the 52 dissected in 3 different experiments proved to be infected. It is possible that these negative results are due to the relatively low prevailing temperature in May and to the slow development of the M.T. parasite at this temperature. It should be noted, however, that the mosquitoes were dissected 10-24 days after feeding, and that no sign of cysts was noted.

Marked variability in the percentage of *Anopheles* infected with *P. falciparum* was noted also during the other months of the year. June gave the highest, and August, September and November the lowest infection rates. Even though the number of *Anopheles* is relatively small, it is difficult to believe that these results are fortuitous.

The results obtained in mass dissections strengthen the significance of the results obtained with experimental infections. In these mass dissections one also obtains a definite seasonal fluctuation. It is of interest, for example, that, although in Palestine the peak of the spring epidemic occurs in July, the incidence of infected mosquitoes in June is either equal to (Table VIIIa) or greater than (Table VIIIb) that in July. In other words, although the number of carriers in July exceeds those in June, the incidence of infected *A. elutus* does not parallel that of carriers. In these natural infections one can only indicate trend, because the results include all types of parasites.

Another interesting point brought out in these studies is the variable susceptibility of *A. elutus* to the different species of *Plasmodium*. *P. vivax* infected 57.9 per cent. of the mosquitoes, *P. falciparum* 27.7 per cent., and *P. malariae* only 8.4 per cent. In other words, it appears that *A. elutus* is twice as efficient a vector of B.T. as of M.T. malaria, and seven times as efficient as of quartan parasites. This may prove of importance in regard to relative incidence of these types of infection. In the light of these results it becomes necessary to compare the efficiency of other vectors under the same conditions. Boyd and Stratman-Thomas (1934) working with *A. quadrimaculatus* obtained results which are almost identical with ours with *A. elutus*; their findings are 52.6 and 67.7 per cent. for *P. vivax*, and 20.9 and 27.0 per cent. for *P. falciparum*. It will be of interest to note the relative susceptibility of a more tropical variety of *Anopheles*, such as *A. sergenti*, to the different species of *Plasmodium*.

Finally, attention should be directed to the specific temperature requirements

of the different species of *Plasmodium*. There is still a lack of systematic studies on this important point. Yet it is apparent that the temperature requirement of each parasite and its inherent rate of development will determine, to some extent at least, the relative prevalence of the various types of malaria in a given locality. The results reported in this connection, though meagre, are of value in helping to fill a gap in our knowledge.

CONCLUSIONS

1. *A. elutus* is most susceptible to infection with *P. vivax*, less so to infection with *P. falciparum*, and least of all to *P. malariae*.
2. It appears that the *Anopheles* possess different degrees of susceptibility to the different species of *Plasmodium* at different months or seasons of the year.
3. The three species of *Plasmodium* have different temperature optima for their development, and different rates of development at a given temperature.
4. Atebrin resembles quinine in its failure to affect M.T. gametes. Atebrin does not prevent infection of *Anopheles* fed on patients under treatment.

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A TECHNIQUE FOR THE INOCULATION OF KNOWN NUMBERS OF SPOROZOITES AS AN AID TO MALARIA RESEARCH

BY

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(Received for publication 28 December, 1936)

In a note entitled 'A New Procedure for Malaria Research,' by James, Nicol and Shute (1927), a method was described whereby the dissected salivary glands containing sporozoites were used for infecting patients, instead of the more natural way of allowing the infected mosquitoes to bite the patient. This method was first used during a visit to a hospital when none of the infected mosquitoes would bite the patient who was awaiting treatment. The difficulty was overcome by dissecting the salivary glands of one of the mosquitoes in Locke's fluid, and, after confirming the presence of sporozoites, by injecting the crushed glands intravenously. This experiment was carried out over 12 years ago, and since that time numerous patients have been infected by this method with *P. vivax*, *P. falciparum* and *P. ovale*, always with success. It has been used extensively in testing the prophylactic value of various drugs, such as quinine, plasmoquin and atabrin. It has also been very useful when taking infected mosquitoes abroad. On one occasion, a batch of mosquitoes infected with *P. vivax* was taken to Jassy, in northern Roumania. Within four hours of arrival many patients were inoculated with sporozoites, the crushed glands of one mosquito being used for each patient.

This technique has recently been further improved, and it is now possible to estimate with some degree of accuracy the number of sporozoites injected. The glands are dissected, in the ordinary way, in a drop of sterile Locke's fluid on a sterile glass slide, and are thoroughly crushed beneath a sterile square cover-slip, of which one edge is left protruding over the side of the slide. The cover-slip is gently removed by lifting it by the protruding corner, thus causing most of the fluid to collect in the middle of the slide. The cover-slip is washed several times with extra drops of Locke's fluid to remove any remaining sporozoites, and the washings are added to the original fluid in the middle of the slide. The fluid on the slide is taken up in a hypodermic syringe, and, by adding further small quantities to the slide, the whole is diluted to exactly 1 c.cm.* The contents of the syringe are then thoroughly mixed.

From this suspension, 0.01 c.cm. is measured in a capillary pipette, spread on a clean slide in as small an area as possible, and, when dry, stained by either Leishman's or Giemsa's technique. By means of an Ehrlich's square eye-piece, the total number of sporozoites in the stained preparation can be counted.*

*The enumeration of the sporozoites is greatly facilitated if a minute drop of blood (not larger than the head of a very small pin) be added to the *original* suspension. The red cells of the stained slide enable one more easily to focus and determine the exact limits of the preparation to be counted.

This number multiplied by 100 gives the total sporozoites per c.cm. of suspension.

A more simple method when counting large numbers is to spread 0.01 c.cm. of the suspension into a square with the sides parallel to the longer edges of the slide. The estimation is made by examining strips across the square and counting all the sporozoites seen. If the number of strips in the whole square is determined, it is easy to calculate the total number of sporozoites in the whole square.

It is advisable to make the square quite small when the number of sporozoites is known to be relatively scanty, and to increase the area of the square when the sporozoites are numerous.

By using the method described above, it has been possible to inoculate patients with known numbers of sporozoites varying from 50 to 760,000. Enormous numbers can be obtained by dissecting a number of glands and collecting them together on one slide, before crushing them beneath a cover-slip.

Attempts to keep sporozoites alive in Locke's fluid outside the body of the mosquito for more than a few hours have so far failed. It is therefore important that as little delay as possible should occur between the dissection of the salivary glands of the mosquito and subsequent inoculation into the patient. The operation can easily be executed in about 10 minutes, even if a number of glands are to be used.

It would appear that in experimental malaria this method has a great advantage over the more usual one of estimating the severity of the infection by the number of infected mosquitoes which bite the patient.

A mosquito which has had numerous oöcysts, all rupturing about the same time, would inject more sporozoites than a mosquito which has only a few oöcysts. But it is not known even approximately how many sporozoites a mosquito injects during the act of biting.

Boyd and Stratman-Thomas (1933) arbitrarily classified the quantitative degree of infection in anophelines into three grades, viz. :—

1. Poor : comprising lots in which not a single positive stomach had more than 30 oöcysts.
2. Fair : an intermediate grade between poor and good.
3. Good : comprising lots in which at least half the positive stomachs had more than 50 oöcysts.

It is reasonable to suppose that one mosquito from group 3 would inject more sporozoites than one of those of group 1. But the actual *numbers* of sporozoites injected by mosquitoes from either of the groups would not be known.

In addition, if a number of mosquitoes (at least with the species *A. maculipennis*) is fed on a good gametocyte carrier at the same time, and is kept under exactly similar conditions, great variation in the number of oöcysts on different individuals occurs ; in one experiment, out of a batch of 30 which fed, 3 were negative, 1 had nearly 800 oöcysts, and the remainder had between a single one and 600 oöcysts.

Jerace (1934) in a recent paper states :—‘ As it is not possible to calculate

accurately the number of sporozoites injected by each *Anopheles*, and as the *Anopheles* used are not always 100 per cent. infected, it follows that if we increase the number of *Anopheles* within certain limits we shall certainly increase the number of sporozoites injected. Allowing for the destruction of a certain number of them, and the possible resistance of some patients, the increase of the dose of sporozoites injected certainly promotes the development of the infection, as can be seen from the fact that the incubation period is shorter and the cases which fail to develop infection are fewer.'

In the study of bird malaria the method described for inoculating known quantities of sporozoites may be useful. If more than about 12 mosquitoes bite a canary or a sparrow at the same time, the loss of blood is considerable, and if malaria develops four or five days later the loss of blood may have so lowered the vitality of the bird that it dies within a few days. On the other hand, a very large and known number of sporozoites ('quantum of sporozoites' (Christophers, 1924)) could be given in a small quantity of fluid without any immediate harmful effects.

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OBSERVATIONS ON *SPIROCHAETA SOGDIANUM* NICOLLE AND ANDERSON, 1928, IN LABORATORY ANIMALS

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The observations outlined in this paper were made on six strains of *Sp. sogdianum*; of these, one was obtained by feeding wild ticks, *Ornithodoros papillipes*, on a guinea-pig, and five were obtained from four infected human beings, all infected in the same cave from which the ticks were collected. Two strains were obtained from the same case (S_5 and S_{11} , fourth and tenth relapses).

As the findings with all six strains were in their general outline rather similar, the experimental data recorded below are based mainly on three strains which were particularly studied with respect to their immunological properties.

The history of these three strains is as follows:—

STRAIN A_9 . 21.1.36. Five c.cm. blood, taken from a human case during the eighth relapse, were inoculated into each of two guinea-pigs, which became infected.

STRAIN S_5 . 11.12.35. Five c.cm. blood, taken from another case during the fourth relapse, were inoculated into two guinea-pigs, which became infected.

STRAIN S_{11} . 10.2.36. Five c.cm., taken during the tenth relapse of the previous case, were inoculated into two guinea-pigs, which became infected.

Both cases were infected by bites of larvae of *O. papillipes* during an inspection of the cave on October 29th, 1935.

The above and other strains were studied in rats, mice, field-mice (*Microtus guentheri*), guinea-pigs, rabbits and dogs.

Thirty-two normal white rats inoculated intraperitoneally with blood containing spirochaetes all became infected. The incubation period lasted from 3 to 9 days, the number of attacks (i.e., the period during which spirochaetes were present in the circulating blood) varied from 2 to 6 days, individual attacks lasted from 1 to 5 days, and the interval between attacks from 1 to 5 days. There was no mortality.

Twelve splenectomized *Bartonella*-free rats were inoculated intraperitoneally. The incubation period varied from 2 to 8 days, the number of attacks from 2 to 5, the duration of attacks from 1 to 14 days, and the interval between attacks from 1 to 4 days. The first attack in splenectomized animals was usually the longest (from 3 to 14 days), and there were relatively long periods (up to 8 days) during which the blood was swarming with spirochaetes. The average

duration of the first attack was 6.5 days, as against 2.5 days in the normal animals.

Seven normal rats were made to swallow blood containing spirochaetes, and all became infected after an incubation period of 4-6 days; the infections ran a normal course.

Heavily infected blood was placed on the backs of 7 young rats (litter-mates 4 days old). The animals were observed for one month and no spirochaetes were found in the blood; but this experiment is not conclusive, for, as will be shown later, an infected rat does not necessarily show a detectable blood infection at any time, although emulsions of the brain are infective for other animals.

Four normal and 4 splenectomized field-mice were inoculated intraperitoneally. Three of the former became infected after an incubation period of 2 days. In 2 animals there was a single attack lasting 1 day, and in the third 2 attacks, each lasting 1 day, with an interval of 1 day. Few spirochaetes were found in the blood during the attacks.

All the 4 splenectomized field-mice became infected after an incubation period of 2 days. In each case there was a single attack lasting 5 days, during the greater part of which period the blood was swarming with spirochaetes.

Three normal mice were inoculated and became infected after an incubation period of 2-12 days. One had a single attack lasting 1 day, and 2 had 3 attacks lasting from 1 to 2 days, with an interval of 3-8 days.

Three adult rabbits became infected after an incubation period of 2 days. Two of the animals had a moderate blood infection during 3 attacks; the third died on the seventh day of the second attack, in which the blood was swarming with spirochaetes. This animal developed an acute iritis.

Two dogs became infected after subcutaneous inoculation. In both cases the incubation period was 5 days. One animal, after inoculation with strain S_5 (described later), had 2 attacks (8 days and 1 day, with an interval of 2 days), with numerous spirochaetes in the blood. After being free from spirochaetes for a period of 44 days, this animal was inoculated with a second strain (A_9) and again became infected.

A third dog became infected after eating two infected guinea-pigs on June 6th, 1936. On July 3rd, the animal was ill and showed photophobia. Numerous spirochaetes were found in the blood. On July 5th, the animal was unable to stand and appeared moribund. The blood was swarming with spirochaetes. A dose of 0.5 gm. of a gold preparation, solganal A, was administered by Dr. R. Freund. On the following day the blood was negative, and the animal made an uneventful recovery.

Guinea-pigs were found to be the most suitable animals for the study of this spirochaete, and our observations were carried out on 290 animals, of which only 2 proved to be refractory. The incubation period in animals inoculated intraperitoneally with infected blood varied from 2 to 34 days; in animals made to swallow infected blood, from 25 to 100 days. One animal was infected

by inoculation of cerebrospinal fluid from an infected rabbit. Six animals were infected by inoculation of fluid from the anterior chamber of infected guinea-pigs. Three out of 4 animals were infected by dropping blood containing spirochaetes into the conjunctiva. The incubation period was 5-6 days. Three animals became infected after ingesting infected blood. Thirty-six animals were inoculated with emulsions of brain from convalescent rats (up to 90 days after recovery), and all became infected, after an incubation period of 3-9 days. Nineteen animals were inoculated with emulsions of brain of convalescent guinea-pigs (up to 130 days after recovery), and all became infected, after an incubation period of from 5 to 90 days. Out of 6 females which gave birth during the period of blood infection, 1 produced a dead foetus, 1 produced a healthy offspring in whose blood no spirochaetes were found after daily examination, 3 produced infected offspring, and 1 produced a litter of two, one with spirochaetes in the blood which survived 2 days, and one which showed no blood infection on daily examination. In one experiment a female was inoculated and became infected while suckling a litter of two, neither of which ever showed a blood infection.

The behaviour of the three strains A_9 , S_5 and S_{11} in guinea-pigs is briefly summarized :—

STRAIN A_9 . Incubation period after inoculation of infected blood 2-7 days.

Individual attacks (periods during which spirochaetes are found in thick smears) last from 1 to 22 days.

Average duration of attack 7 days.

Interval between attacks 1-3 days.

Number of attacks 1-3.

Average number of attacks per guinea-pig 1.7.

Duration of disease (from first appearance to final disappearance of spirochaetes from the blood) 1-22 days.

Average duration of disease 14.4 days.

Mortality 5.7 per cent. (2 out of 35 uncomplicated cases).

STRAIN S_5 . Incubation period after inoculation of infected blood 2-34 days—in the majority of cases 4-6 days.

Individual attacks last from 1 to 23 days.

Average duration of attack 6 days.

Interval between attacks 1-4 days.

Number of attacks 1-5.

Average number of attacks per guinea-pig 2.

Duration of disease 12-34 days.

Average duration of disease 19.2 days.

Mortality 37 per cent. (11 out of 30 uncomplicated cases).

STRAIN S_{11} . Incubation period after inoculation of infected blood 2-6 days.

Individual attacks last from 1 to 19 days.

Average duration of attack 4 days.

Interval between attacks 1-3 days.

Number of attacks 1-6.

Average number of attacks per guinea-pig 1.8.

Duration of disease 11-32 days.

Average duration of disease 15 days.

Mortality 46 per cent. (17 out of 37 uncomplicated cases).

When first isolated, this strain was very virulent (9 out of 14 guinea-pigs died), but it became milder after passage through a rat.

The variation in the incubation period depends not only on the number of spirochaetes inoculated, but also on individual differences. In one experiment two guinea-pigs were inoculated simultaneously with the same quantity of blood: in one case the incubation period was 34 days, in the other only 5 days.

In many cases the cause of death is extensive haemorrhage into the peritoneum, lungs and pleura, but in some cases no gross anatomical cause of death can be found. Fatal haemorrhages into the peritoneum are, as Brumpt pointed out, in some cases due to rupture of the enormously enlarged spleen. Haemorrhages may occur in any site (muscles, joints, brain, intestine), but they are most common in the peritoneum, pleura and lungs. In one case death was due to a haemorrhage in the brain, in another, after 4 days' infection, to a haemorrhage in the heart muscle. A fatal haemorrhage may occur not only during the infection, but also within the first 24 hours after the blood has become free from spirochaetes.

Apart from the above-mentioned haemorrhages, the most striking findings are an enormously enlarged spleen (up to 10 times the normal), infarcts in the spleen and lungs and enlarged adrenals. Infarcts of the spleen and lungs probably occur frequently in infections fatal or otherwise, for they are found in many animals sacrificed during infection (15 out of 33 with infarcts in the spleen, and 12 out of 33 with haemorrhages in the lungs). Exudation through the capsule and the formation of a fine layer of fibrin on the surface of the spleen are common. As a result of this process, adhesions between the spleen and neighbouring tissues, and displacement of this organ in cured animals, are common, e.g., the spleen may become adherent to the diaphragm or intestines and displaced from its natural position.

Tissues were studied after fixation in formalin and staining by Dobell's method for the demonstration of spirochaetes. During infections spirochaetes are found in all tissues, both in blood vessels and in tissue spaces, but they appear to be most numerous in the liver.

The following picture is constructed from findings in fatal cases and in animals sacrificed in all stages of infection.

Liver. The histological picture varies according to the stage of infection. In all stages spirochaetes are found both in the vascular system and among—but not in—the cells of the parenchyma. It is quite easy to follow the escape of spirochaetes from the capillary into the interstitial tissue.

In the earlier stages there is no particular change, beyond the presence of spirochaetes. Later (when the blood infection is heavy), there is an intense phagocytic activity on the part of the Kupffer cells, but this activity is *largely indiscriminate*. The Kupffer cells ingest red cells and all types of leucocytes, and only a few spirochaetes may be ingested, even though the liver is intensely infected. A day after the blood infection has disappeared, no spirochaetes are

found in the liver. Possibly the activity of the Kupffer cells towards the end of the infection, instead of being indiscriminate, has become specific towards the spirochaetes, which are ingested and rapidly destroyed. The latter is a reasonable conjecture supported by serological evidence, although the final process of the disappearance of parasites from blood and tissues was not followed histologically, because it was never possible to predict the termination of an infection.

In some (but not all) fatal infections large globules of fat are found in the liver cells.

Spleen. Spirochaetes are numerous in infarcts and in small extravasations of blood. In the majority of cases they are relatively few in all stages of infection among the cells of the spleen pulp.

An interesting phenomenon was noted in very severe cases, and the following description is based on the composite findings in a number of spleens showing various stages of the process. The Malpighian bodies either whole or in parts degenerate, and the nuclei stain feebly. In cases where only a part of the Malpighian body is affected, the boundary between affected and healthy tissue is very clear (Pl. III, fig. 3). At this stage few or no spirochaetes are found in the involved tissue. Later, the nuclei of the degenerated area fragment, and spirochaetes are found among the broken-up cells. The area is finally invaded by large numbers of polymorphs, till it becomes practically converted into an abscess (Pl. III, fig. 4). This area of necrosis swarms with spirochaetes, in marked contrast to the surrounding macrophages among which spirochaetes are relatively few.

Lungs. As in all other tissues, spirochaetes are found in large numbers in areas of infarcts and haemorrhages, and occur in varying numbers in the interstitial tissues round the alveoli. A few spirochaetes make their way into the alveoli.

Brain. Haemorrhages in the brain from the arterioles, small veins and capillaries may occur during the infection, or shortly after the spirochaetes have disappeared from the blood (Pl. III, figs. 1, 2). Small individual haemorrhages in the brain are probably not rare and may show no symptoms, for they may be found in animals sacrificed during the infection.

During the infection parasites are found in the blood system; they often appear to be in close apposition to the endothelial cells of capillaries. A few spirochaetes are partly inside and partly outside the capillaries, i.e., in the act of escaping into the surrounding tissue. In the brain substance proper they can be found during the infection, generally in the neighbourhood of capillaries, but they are never present in large numbers except in haemorrhagic areas. After spontaneous cure it was not possible to find spirochaetes in sections, although their presence can always be demonstrated by inoculating emulsions of brain into clean animals.

Two animals died with intense congestion of the brain and meninges.

IMMUNITY

Five animals in our series had a slight transient infection (a few spirochaetes in a thick drop during 24 hours). Of these, one was successfully reinoculated and showed the normal course of infection.

An emulsion of brain of another of these animals, one month after the blood was free from spirochaetes, was inoculated into a rat, which showed no blood infection during an observation period of 2 weeks. The brain of the rat was then emulsified and inoculated into a guinea-pig; after an incubation period of 9 days, spirochaetes were found in the blood of the inoculated animal. It thus appears that slight transient infections, and even infections too slight to be detected by examination of thick drops, leave a residual brain infection.

Apart from slight transient infections, an infection spontaneously cured was found to confer a solid immunity against the original strain during an observation period lasting up to 6 months. The result is the same whether the second inoculation is made from a rat or a guinea-pig in any stage of infection.

This immunity is strictly strain-specific, and in numerous experiments there were only two exceptional instances where an attack of S_{11} conferred immunity against A_9 . Nevertheless, an attack of one strain conferred some degree of protection against other strains, which manifested itself in a shortening of the duration of the disease and the reduction in the mortality.

The examples shown in Table I will suffice.

As already stated, strain S_5 in previously untreated animals produced a mortality of 37 per cent., the average duration of individual attacks was 6 days, and the number of attacks per animal was 2. In animals spontaneously cured from A_9 and then inoculated with S_5 , the mortality was reduced to 1 out of 8, the average number of attacks was 2, and the average duration of individual attacks was 2.5 days.

It is interesting to note that this partial protection is less between the two strains S_5 and S_{11} , isolated from the same human case, than between either of these strains and A_9 , isolated from another case.

It should be emphasized that virulence plays no great part in cross-immunity, e.g., the relatively virulent S_5 produced no immunity against the much less virulent A_9 , and the absence of cross-immunity therefore obviously depends on differences in the antigenic constitution of the various strains.

Splenectomy. As previously stated, splenectomy before infection made a profound difference in the type of infection produced in rats and field-mice. In 2 guinea-pigs splenectomized before inoculation the infection appeared after an incubation period of 2 days, and ran a normal course. A month after recovery they were reinoculated and showed a slight transient infection lasting 1 day. A month later they were again reinoculated and again showed a transient infection. Further reinoculations gave negative results.

The effect of splenectomy carried out up to one month after recovery is variable. In one animal a slight relapse lasting 1 day occurred 6 days after

TABLE I

Guinea-pig no.	Strain producing first infection	Interval after first attack	Strain producing second infection	Results
28	A ₉	6 months	S ₅	2 attacks, 3 days and 1 day. Interval 2 days
59	A ₉	5 months	S ₅	3 attacks, 1 day, 3 days and 1 day. Intervals 1 and 3 days
60	A ₉	5 months	S ₅	1 attack, 5 days
63	A ₉	5 months	S ₅	1 attack, 5 days
64	A ₉	5 months	S ₅	2 attacks, 5 days and 1 day. Interval 3 days
76	A ₉	59 days	S ₅	Fatal infection, lasting 6 days
98	A ₉	3 months	S ₅	2 attacks, each 1 day. Interval 7 days
115	A ₉	1 month	S ₅	3 attacks, 1, 2 and 4 days. Intervals 3 and 1 days
48	S ₅	4 months	A ₉	2 attacks, 2 and 1 days. Interval 1 day
81	S ₅	5 months	A ₉	2 attacks, 5 and 1 days. Interval 2 days
9	S ₅	46 days	S ₁₁	2 attacks, 2 and 1 days. Interval 2 days
22	S ₅	43 days	S ₁₁	2 attacks, 6 and 1 days. Interval 1 day
23	S ₅	35 days	S ₁₁	Died after 4 days' heavy infection
32	S ₅	32 days	S ₁₁	2 attacks, 1 and 2 days. Interval 2 days
58	S ₁₁	36 days	S ₅	Fatal infection, lasting 8 days
91	S ₁₁	37 days	S ₅	Fatal infection, lasting 2 days
92	S ₁₁	40 days	S ₅	1 attack, 3 days
101	S ₁₁	30 days	S ₅	2 attacks, 3 and 2 days. Interval 2 days
36a	S ₁₁	51 days	A	2 attacks, 7 and 1 days. Interval 1 day
38	S ₁₁	51 days	A ₉	2 attacks, 6 and 1 days. Interval 2 days
35	S ₁₁	50 days	A ₉	—
34	S ₁₁	50 days	A ₉	—

the operation, and the animal was subsequently immune to further inoculations of the same strain. In four other animals no relapse occurred after the operation. Two of these proved resistant on reinoculation, and two showed a slight infection when reinoculated one month after splenectomy.

Immunity After Mixed Infections. Animals inoculated simultaneously with two strains, or inoculated with a second strain in the course of an infection with one strain, became immune to both strains but not to a third strain; e.g., animals inoculated in the above manner with S_5 and A_9 became immune to S_5 and A_9 but not to S_{11} .

If, however, the infections are not simultaneous, and the second strain is inoculated after recovery from the first strain, the animal thus treated becomes immune not only to the two strains employed, but also to a third strain; e.g., if an animal recovers from S_5 and is then inoculated with A_9 and again recovers, it becomes immune to A_9 , S_5 and S_{11} . This phenomenon was observed frequently, and examples are shown in Table II.

TABLE II

Guinea-pig no.	First strain	Interval after spontaneous cure	Infected with second strain	Interval after spontaneous cure	Infected with third strain	Result
1	Wild tick strain	27 days	S_5	35 days	S_{11}	—
9	S_5	46 days	S_{11}	30 days	A_9	—
22	S_5	43 days	S_{11}		A_9	—
31	A_9	35 days	S_{11}	23 days	S_5	Slight transient infection lasting 2 days
32	S_5	32 days	S_{11}	32 days	A_9	—
36a	S_{11}	50 days	A_9	32 days	S_5	—
64	A_9	57 days	S_5	32 days	S_{11}	—

Similar experiments on rats (with other strains) gave identical results.

The above observations indicate that, in spite of their differences, all the strains obtained from the same cave gave some degree of cross-protection, and the additive effect of two separated infections with different strains are sufficient to produce an immunity to any third strain. It should, however, be noted that

any combination of separated infections of the above strains failed to produce immunity to a strain obtained from a different part of Palestine.

The individual strains used in mixed infections, either simultaneous or successive, can usually be separated by inoculating emulsions of brain of a recovered animal into others rendered immune to a single strain; e.g., if emulsions of the brain of an animal recovered from A_9 and S_5 are inoculated into an animal immune to A_9 , the S_5 strain is recovered; and, in an animal immune to S_5 , the A_9 strain is obtained. It is therefore evident that all strains inoculated into a single animal remain as individual strains in the brain. (The above experiment is usually, but not uniformly, successful.)

Serological Observations. The three strains S_5 , S_{11} and A_9 were compared serologically. The following technique was used. Sera from recovered guinea-pigs or human beings were made up in various dilutions up to 1:5,000, in test-tubes about 0.5 cm. in diameter, and were tested against homologous and other strains of spirochaetes. Defibrinated blood heavily infected with spirochaetes was used as antigen; 0.1 to 0.2 c.cm. (according to the number of spirochaetes) was added to each tube. Series of tubes with serum and antigen were kept at 30° C., and parallel series at 37° C. The latter were examined after 2 hours, and again on the following day. The former were examined after standing over night. All manipulations should be carried out with aseptic precautions. Examinations were made in fresh cover-slip preparations, and when necessary in stain preparations. Convalescent sera from 9 guinea-pigs and 2 human beings were examined.

This proceeding was adopted because the one recommended for *Sp. obermeieri* (i.e., examining fresh cover-slip preparations after half an hour) was not found satisfactory, since no serological phenomena of note were observed till 2 hours at 37° C. The above method has the additional advantage of permitting observations both on agglutination and on certain serological phenomena associated with leucocytes which are probably of wide application.

It was easy to differentiate the various strains by means of agglutination tests. Thus, one serum, from a guinea-pig convalescent from S_{11} , agglutinated S_{11} to 1:5,000, S_5 to 1:200. The sera of recovered animals agglutinated the homologous strain from a titre of 1:2 to 1:5,000. Auto-agglutination in the control was observed on several occasions but is sufficiently rare not to interfere with the general results. In addition to agglutination, homologous sera immobilized a varying number of spirochaetes both in the agglutinated masses and free spirochaetes apart from these masses. Immobilized spirochaetes were often swollen and showed small vesicles attached to their surface—obviously degeneration phenomena. Normal human or guinea-pig serum may have an agglutinating titre up to 1:10, and occasionally up to 1:20. Under the influence of immune sera, the spirochaetes attached themselves to leucocytes (polymorphs, lymphocytes and mononuclears). The attachment is by one extremity only, never along the longitudinal surface of the spirochaete. Waves of contraction pass along the

body of the spirochaete, which may bore through the wall of the polymorph or large mononuclear till it lies completely embedded in the protoplasm of the leucocyte (Pl. II, fig. 1). A large number of spirochaetes may attach themselves to a single cell, and in cases where considerable numbers have penetrated into a single cell the protoplasm is destroyed and only the nucleus remains. In cases where the protoplasm of the leucocyte is destroyed, spirochaetes attach themselves by one extremity to the nucleus. Frequently clumps of leucocytes are found covered by a matting of spirochaetes, attached to their protoplasm or their nuclei (Pl. II, figs. 4, 5). The above process is independent of agglutination, although a serum may show both phenomena to a high titre. This is proved by the fact that only one or two individual spirochaetes may be attached to a single cell.

Individual specific sera show this phenomenon in a titre varying from 1 : 2 to 1 : 4,000. As with agglutination, a high titre serum for one strain shows this phenomenon in a low titre for a second strain.

In the above specific reaction, leucocytes are entirely passive in experiments carried out at 30° C., and some of the leucocytes involved are denuded of protoplasm either through the action of the spirochaetes or because of the conditions *in vitro*.

At 37° C. the attachment of spirochaetes to leucocytes and the presence of spirochaetes in the plasma of polymorphs is well marked after 2 hours, but it is not possible in this case to distinguish between the specific action of the serum and the autonomic action of the leucocytes. Probably both factors play a part, because there are more spirochaetes embedded in the protoplasm of polymorphs at 37° C. than at 30° C. At 37° C. some of the spirochaetes contained in the polymorphs are enclosed in vacuoles—a reaction not seen at a lower temperature. At least some of the phagocytic activity at 37° C. is non-specific and indiscriminate, for red cells are occasionally ingested, as many as four being found in a single polymorph. By comparing the findings at both temperatures, it is possible to analyse the specific effects of the serum from the autogenous activity of the leucocytes.

A phenomenon similar to the one described above was discovered and illustrated perfectly by Himmelweit (1933), in the case of immune serum from fowls recovered from infections of *Sp. gallinarum* and macrophages from tissue cultures of fowl spleen. This author found that, when spirochaetes are added to a culture of spleen of an immune bird, they begin to bore into macrophages after 1 hour, and in cultures of normal spleen only after 30 hours. On adding immune serum the spirochaetes quickly begin to bore into macrophages grown from the spleen of normal birds. The passive rôle of the macrophages in this process is emphasized.

This author's observations are confined to macrophages, and he did not work with polymorphs. His sera were active in titres of 1 : 2 to 1 : 20, while some of our sera showed a far higher titre. The action of the immune sera in

both cases is obviously due to a factor related to opsonins and bacteriotropins, and Himmelweit named this factor in the immune serum 'spirochaetotropin.' It is interesting to note that the same mechanism applies to phagocytic cells of such divergent character and origin as the polymorphs and the macrophages. The phagocyte is in both cases passive, and the spirochaete, activated by the immune serum, actively bores its way into the protoplasm of the phagocyte, being apparently attracted to the nucleus of the latter.

Of two available human sera examined, one (against A_9) was active only in a titre of 1 : 2, and the other in a titre of 1 : 4,000 against S_{11} and 1 : 200 against S_5 . In the case of both human and guinea-pig sera, inactivation lowers the titre (in one case from 1 : 4,000 to 1 : 200) but does not abolish the above phenomenon.

It is probable that agglutination, immobilization and attachment to leucocytes are three independent phenomena. A serum may show a high titre for agglutination and produce little immobilization, and in sera which show both phenomena many individual leucocytes not included in agglutinated clumps are immobilized.

The prophylactic and curative properties of immune sera were tested on 11 guinea-pigs. Homologous sera of immune animals and human convalescent sera in relatively large doses (1–1.5 c.cm.) did not have the slightest prophylactic value when inoculated together with spirochaetes in 6 guinea-pigs, and did not influence the course of the disease when inoculated during the period of blood infection (in 5 guinea-pigs), even when the serum employed had an agglutinating titre of 1 : 5,000. None of our sera, human or guinea-pig, showed any marked lytic action on the spirochaetes.

The above-mentioned serological phenomena may be of value in establishing immunity in the first place. During the whole course of infection, some phagocytosis takes place, e.g., in the spleen, where the number of macrophages is so large that contact between macrophages and spirochaetes is inevitable. In individual animals sacrificed while the blood is swarming with spirochaetes, it may be difficult to find parasites in the spleen outside the lesions mentioned in a previous part of this paper. When spirochaetes are swarming in the bloodstream, it is not difficult to find polymorphs with ingested parasites, and here again the occasional contact between a number of leucocytes and spirochaetes is inevitable; but such occasional phagocytosis cannot appreciably influence the number of rapidly multiplying spirochaetes. The immunological value of a specific factor which inevitably brings spirochaetes into contact with phagocytes, many of which (such as the Kupffer cells) are stationary, is obvious; but since it is impossible either to cure or to establish a passive immunity by means of immune serum, and since immunity may persist after a marked diminution of all these factors, or even in cases where the titre was low (1 : 20) from the beginning of convalescence, they are obviously not the main determining course in the maintenance of immunity. The latter is to be sought elsewhere—probably

in an acquired capacity of the macrophages of destroying spirochaetes more quickly than the latter can multiply.

SUMMARY

Spirochaeta sogdianum Nicolle and Anderson (1928) was found to be infective for mice, rats, field-mice (*Microtus guentheri*), guinea-pigs, rabbits and dogs. Animals were infected by intraperitoneal and subcutaneous inoculation, by the oral route and through the conjunctiva. In splenectomized rats and field-mice the infection was found to be heavier and ran a longer course than in normal animals.

The fluid from the anterior chamber of the eye was found to be infective.

The spirochaete can pass through the placenta of guinea-pigs.

The morbid anatomy of the infection in guinea-pigs is described.

The spirochaete is neurotropic.

Even in guinea-pigs and rats which have passed through a slight transient infection, the spirochaete persists in the brain. In an animal with a slight infection not detected in thick drops, the presence of spirochaetes in the brain was demonstrated by inoculation into a susceptible animal.

Guinea-pigs which have passed through a moderate or severe infection are immune to reinfection with the same strain.

This immunity is strictly strain-specific.

Two strains isolated from the same human being (fourth and tenth relapse) could be distinguished by cross-agglutination and cross-immunity tests.

Three strains from two human beings, infected at the same time in the same place, were studied particularly. A concurrent mixed infection of two of the above strains did not protect against the third strain, but two separated infections of two strains protected against the third.

Convalescent sera, in addition to agglutinating and immobilizing homologous spirochaetes, contain a specific factor which causes spirochaetes to attach themselves by one end to lymphocytes, mononuclears and polymorphs, and to the nuclei of these cells after disintegration of their protoplasm. This factor causes the entrance of spirochaetes into polymorphs in the complete absence of phagocytic activity on the part of the latter.

Convalescent sera have no curative or prophylactic value.

ACKNOWLEDGEMENT.—We have to thank Dr. L. Doljansky, of the Cancer Institute of the Hebrew University, for microphotographs.

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PLATE II

EXPLANATION OF PLATE II

- Fig. 1. A coil of spirochaetes in a polymorph. Convalescent guinea-pig serum 1 : 20 at 30° C. (\times 1,400.)
- Fig. 2. A spirochaete inside a polymorph and several attached spirochaetes. Convalescent human serum 1 : 2,000 at 37° C. (\times 1,400.)
- Fig. 3. Numbers of spirochaetes attached to a single polymorph. Human convalescent serum 1 : 2 at 30° C. (\times 1,400.)
- Fig. 4. Large numbers of spirochaetes attached to leucocytes. Guinea-pig convalescent serum 1 : 20 at 30° C. (\times 1,400.)
- Fig. 5. Large numbers of spirochaetes attached to a group of leucocytes and nuclei of leucocytes. A number of spirochaetes have penetrated into polymorphs. Guinea-pig convalescent serum 1 : 20 at 30° C. (\times 1,600.)

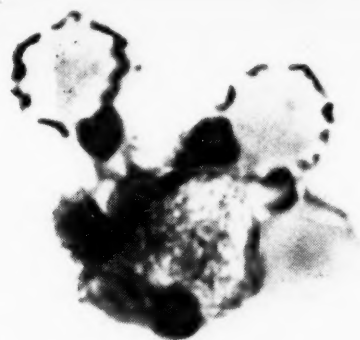


Fig. 1

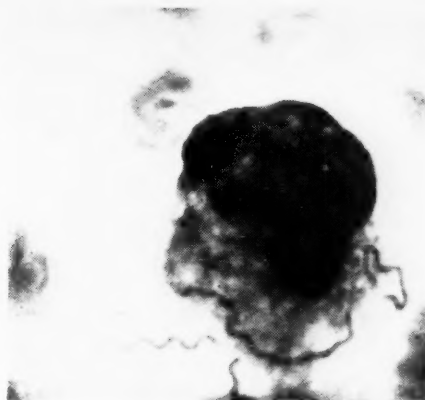


Fig. 2



Fig. 3

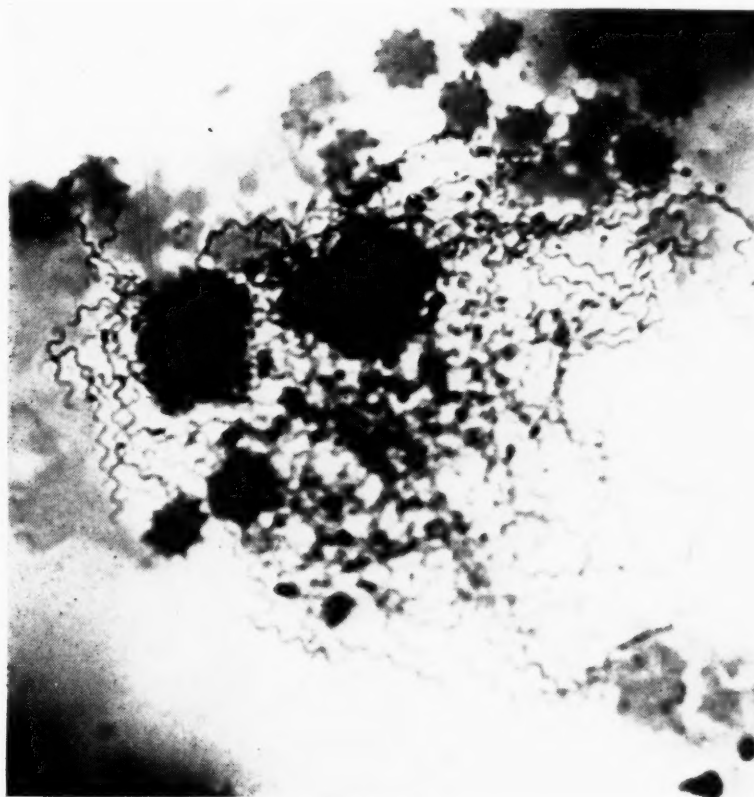


Fig. 4

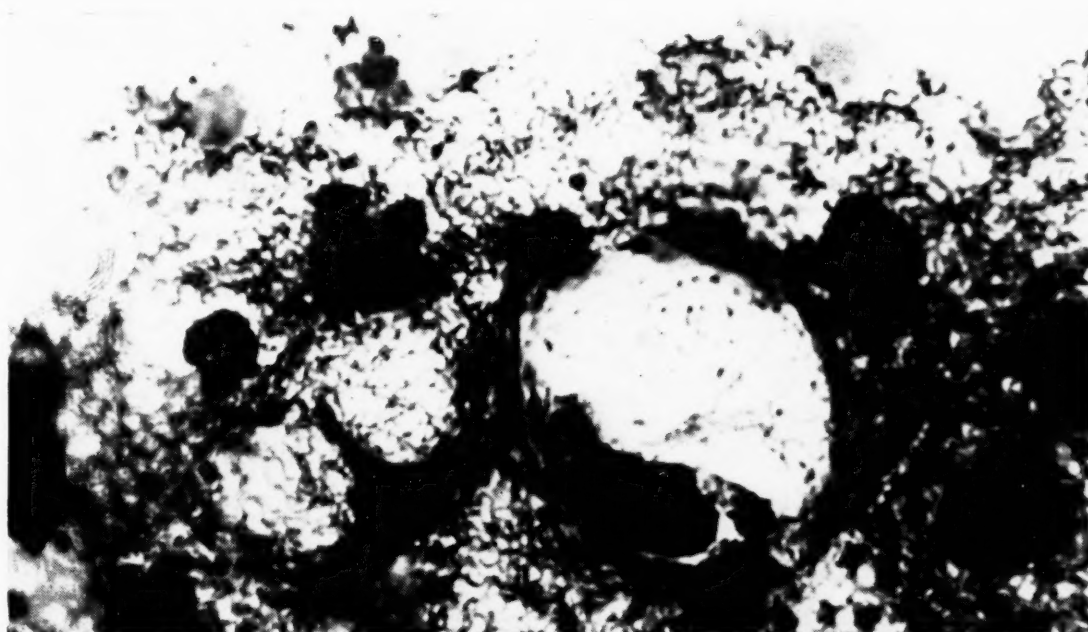


Fig. 5

EXPLANATION TO PLATE III

Figs. 1 and 2. Fatal haemorrhages localized and diffuse in the brain of a guinea-pig within 24 hours after the blood was free from spirochaetes. ($\times 38\frac{1}{2}$.)

Figs. 3 and 4. Stages in degenerative process in Malpighian follicles of spleen in guinea-pigs. Fig. 3, early stage; fig. 4, late stage, showing necrosis, breaking up of cells and invasion by polymorphs. ($\times 200$.)

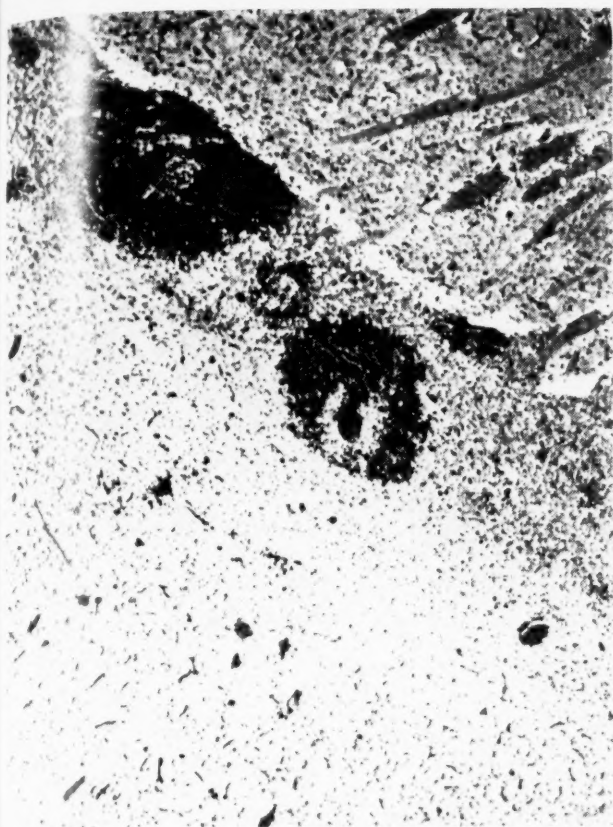


Fig. 1

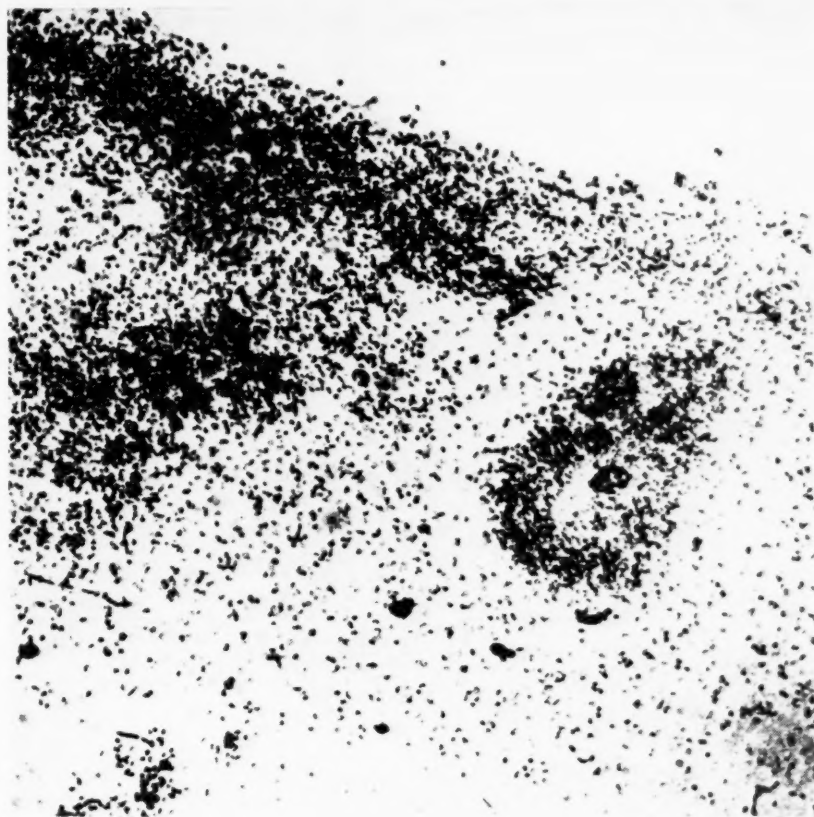


Fig. 2

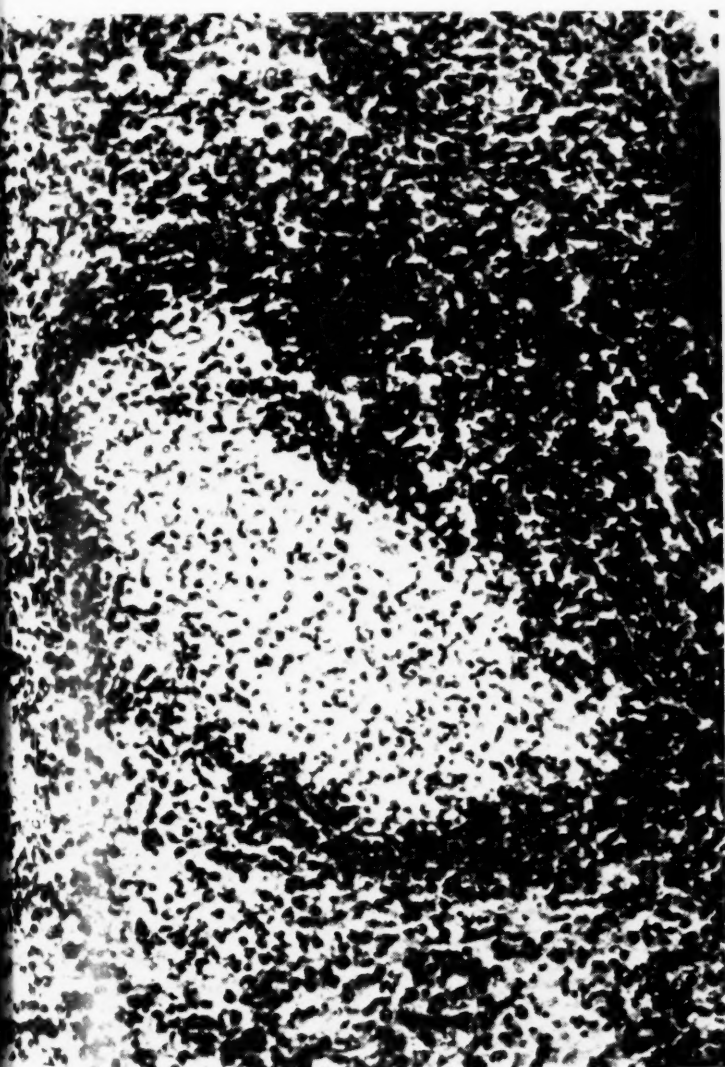


Fig. 3

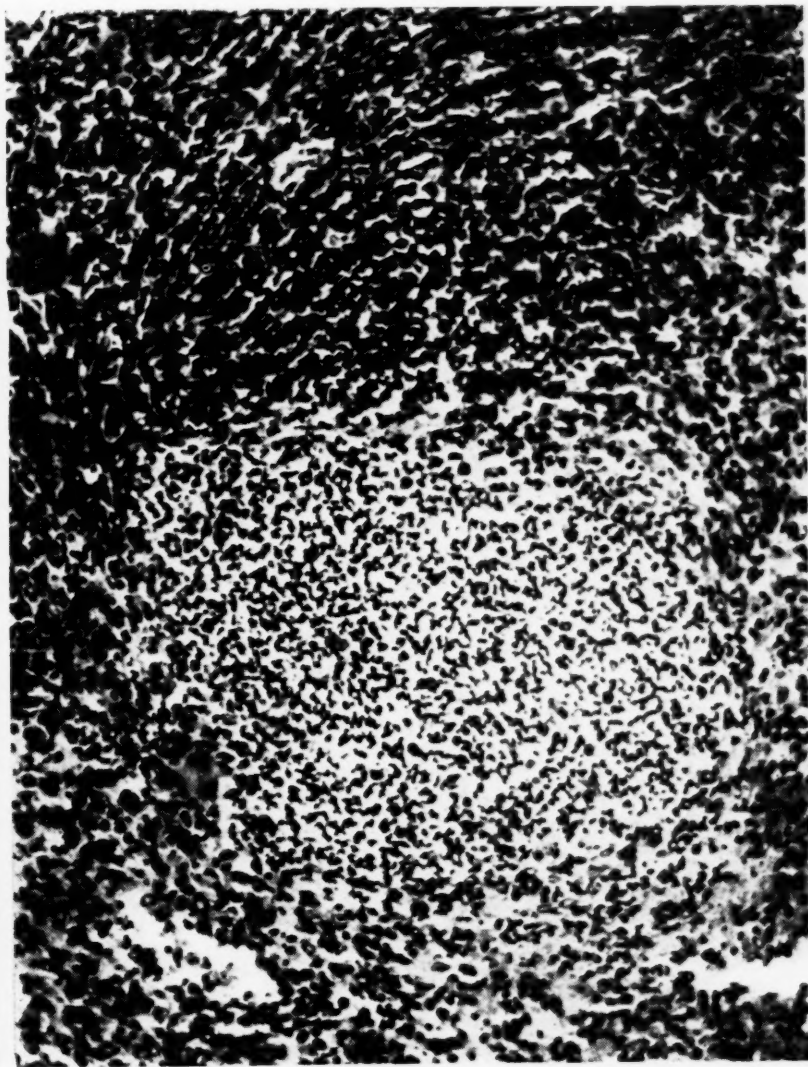


Fig. 4

ANOPHELES FUNESTUS AND ITS ALLIES IN KENYA

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(Received for publication 4 February, 1937)

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INTRODUCTION

In a preliminary communication, one of us (A. M. E., 1936) recorded the results of examining larvae of the *funestus* series collected from various parts of Kenya and sent by the other author (C. B. S.). The great majority of these larvae agreed with the type form of *funestus*, but a number resembled those of *A. rivulorum* Leeson or *A. lesoni* Evans, and a single specimen appeared to be *A. funestus* var. *confusus* E. and L. Further, among the *funestus*-like larvae a good deal of variation was observed in the colour and shape of the main tergal plates. These findings showed the necessity for further investigation of the subject by one who could devote uninterrupted time to rearing and studying material of the various forms from isolated larvae. In order that these and other investigations on the systematics of some of the Kenya anophelines could be carried out, the Leverhulme Research Committee, to whom we wish to express our best thanks, made a grant to one of us (A. M. E.), who was therefore able to spend nearly five months in the Colony in 1936. Grateful acknowledgement must also be made to Professor W. S. Patton, Professor W. Yorke and the Council of the Liverpool School of Tropical Medicine, who kindly granted leave of absence. Some of the results of the investigations carried out have already been published (1936) jointly with Dr. P. C. C. Garnham, who collaborated in the work at Kisumu.

It is now possible to determine anophelines of the *funestus* series from larval material alone, and to distinguish between ♀♀ of *A. funestus* type form and those of other members of the series in Kenya.

Anopheles funestus Giles type form

The preliminary statement that this was evidently the greatly predominating form of the series in Kenya has been amply confirmed. In many localities it is the only member of the series collected, and in most places where *A. rivulorum* and *A. lesoni* have been found they occur in relatively insignificant numbers. A considerable amount of material was bred from isolated larvae collected from different parts of the Colony, from the coast to the Kavirondo Gulf, and the adults from widely separated localities were found to agree in external and morphological characters with those of Sierra Leone (type locality) material.

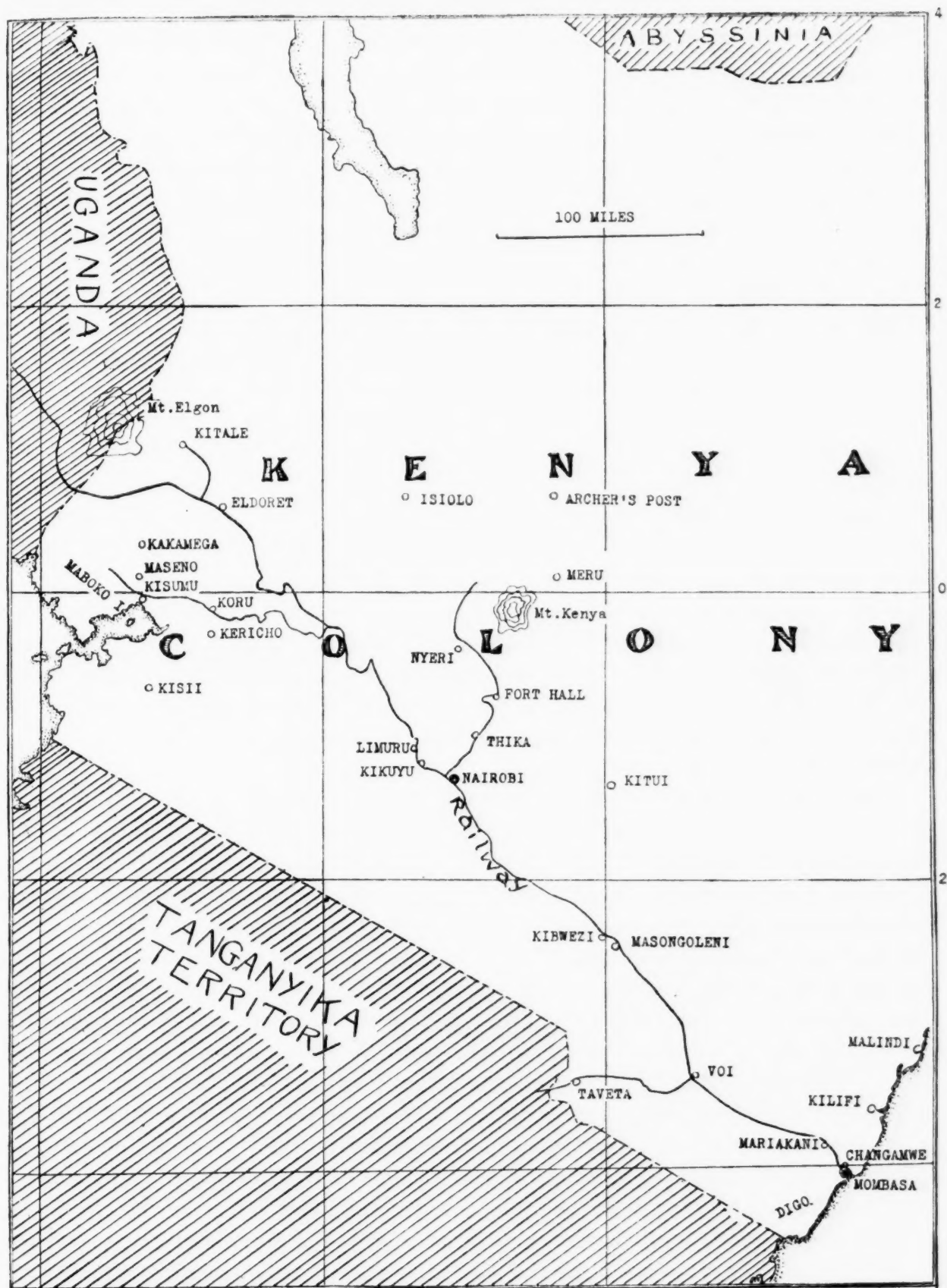
By adopting a rapid standard method of mounting the pharynx, it was found that the length of the post-armature ridges gave a reliable means (if occasional doubtful mounts were excluded) of distinguishing the ♀♀ from those of other members of the series.

DISTRIBUTION (see map, p. 107). The distribution of *A. funestus* in Kenya has been given by one of us (C. B. S., 1932), and there is little doubt that all or almost all the records refer to the type form. Material determined as this form during the present investigation was collected in the following localities (the position of most of these is shown in the map, p. 107): Kisumu, Maboko Island, Kibigori, Koru, Kakamega, Kitale, Fort Hall, Kitui, Taveta, Masongoleni, Mariakani, Kisauni and Chagamwe (mainland near Mombasa), Digo district, Kilifi, Malindi. In many of these districts the species was very common.

The larvae obtained from wells and indoor tanks at Malindi (C. B. S., 1936) were those of the type form; the highly unusual breeding places in this old coastal town are illustrated in Pl. IV at the end of the present paper.

In three districts where larvae of *A. rivulorum* or *A. lesoni* had been found, special surveys were made, which revealed that *A. funestus* type form vastly outnumbered the other member of the series present, which was much more restricted in its breeding places, and in one case (*A. lesoni* at Fort Hall) had evidently been eliminated from the district by control measures.

HABITS OF ADULTS. *A. funestus* type form is obviously the only *funestus*-like anopheline which normally frequents huts or houses in most, if not all, parts of the Colony in which the series occurs. Owing to the lack of external differences between ♀♀ of *A. rivulorum* (and certain specimens of *lesoni*) and *A. funestus*, some doubt existed as to the exact identity of many of the specimens of *A. funestus* formerly recorded from human dwellings. The pharynxes of several ♀♀ caught indoors all proved to be *A. funestus* type form. These specimens were taken at random from four widely separated localities in the Colony. Further, it has been shown that at Kisumu (Evans and Garnham, 1936) this form and *A. costalis* (*gambiae*) were the only anophelines found in native huts near to a *funestus* swamp in which *A. rivulorum* was also breeding prolifically. No such convenient breeding place of *A. lesoni* has been available for study of the house-frequenting habits, but large collections of the *funestus* series taken in huts in different parts of the Colony have been examined, and no ♂♂



Sketch-map showing places in Kenya referred to in the present paper and also by one of the authors (Symes, 1932) in recording the distribution of *Anopheles funestus*.

or characteristic ♀♀ of *leesoni* have been found among them. If any considerable proportion of ♀♀ of that species had been present, some, at least, of them would have been detected by the third vein (pale area, when present, situated in the middle of the vein and often occupying much more than $\frac{2}{3}$ of the vein).

RELATION TO MALARIA. As *A. funestus* type form is obviously the common hut-frequenting member of the series in Kenya, the records of natural infectivity (Symes, 1932) must apply to this form.

VARIATION IN SIZE AND SHAPE OF TERGAL PLATES. Measurement of the main tergal plates of numbers of larvae and pelts from different parts of the Colony and from West Africa has shown that there is a great deal of variation in the size of the plates and also in the proportion of depth to breadth. The largest (widest) plate on segment V examined was that of a larva from the Digo district of Kenya, which measured 0.57 mm. across; and the minimum width seen among larvae from the same locality was 0.37 mm. These were also the limits in width of this plate seen among normal *funestus* larvae caught in Kenya as a whole. In the Sierra Leone larvae examined, the plate was usually narrower than the average width in Kenya specimens, but no larvae were seen in which the width was below the minimum observed in East African larvae.

The size variation in the plates is not merely due to variation in the size of all parts of the larva. The fronto-clypeal ratio (length of fronto-clypeus divided by width of plate on V) was found to vary considerably. In two large-plated pelts measured the ratio was less than 1 (0.93 and 0.96), and the highest ratio found was 1.26 (plates small). Larvae from the same locality showed marked differences in this respect.

ABNORMAL VARIATION IN TERGAL PLATES OF LARVAE RAISED TO FOURTH STAGE FROM EGGS. Among the batches of larvae raised from eggs by one of us at Kisumu (see Evans and Garnham, 1936), it was surprising to find series in which a considerable proportion of larvae had the paired platelets isolated from the main plate (on one side at least) on all the segments. In extreme cases the platelets were all detached, the larvae thus resembling those of *A. funestus* var. *confusus*. Such specimens differed markedly, however, from the larvae of var. *confusus*, in having the main plates greatly reduced in width as well as in depth, and hair 'O' was sometimes situated well off the main plate, as in *A. leesoni*. In the variety the main plates, though relatively shallow, are broad (as broad as large-plated larvae of the type form). In contrast to this, the plates in the atypical raised larvae of *funestus* were abnormally small; the fronto-clypeal ratio was higher in all cases than in any caught larva from Kisumu measured. It should be stated that some series showed great irregularity in the character, and it was frequently seen that even on segments IV-VI one platelet was attached to the main plate, the other free. There was also a considerable amount of variation among individual larvae in some of the broods. These observations suggested that the partial or complete isolation of the platelets was probably due to reduction of the main plates. Comparative measurements were therefore

made, and it was found that, where the isolation of the platelets was complete or almost so on IV-VI, the reduction of the main plates, as indicated by fronto-clypeal ratio, was greatest. Further, the fact that, among more than 200 larvae caught at Kisumu, only 2 showed a marked tendency to isolation of the platelets, and that in neither of these were the platelets completely off on V and VI, makes it obvious that the abnormal condition was brought about in some way by the conditions under which the larvae were reared.

This observation is of some importance, because it shows that the varietal characters of larvae raised from the egg in the laboratory may be changed to some extent. Too much reliance should not be placed on the characters of such artificially raised larvae, unless checked by naturally occurring larvae.

Anopheles funestus var. **confusus** Evans and Leeson

Since the discovery among Kenya material of a single larva referred to by one of us (A. M. E., 1936), no specimens have been found with the characters of this variety.

In a single larva (pelt) among 83 collected at Fort Hall in September, 1936, the accessory paired plates were isolated from the main plates on all segments, but the main plates were smaller than in any other *funestus* larvae in this collection. This specimen is therefore comparable to the abnormal larvae raised from eggs at Kisumu. Two other Fort Hall larvae approached this condition, but one or both platelets were attached to the main plate on some of the segments. These larvae also showed reduction of the main plates, and it is obvious that all three specimens are to be regarded as abnormal examples of *A. funestus* type form. A possible explanation is that these larvae may have been reared in the laboratory from a very early stage; I have never seen the condition when examining freshly caught fourth stage larvae of *funestus* in Kenya.

In the larva recorded in 1936 the tergal plates are broad, well developed and convex posteriorly as in *A. funestus* var. *confusus*. The variety is therefore to be regarded as almost certainly present, but extremely localized and rare in Kenya.

Anopheles rivulorum Leeson

This species can now be recorded from the Kisumu district in the west; from Mariakani, about 20 miles inland from Mombasa; from the Digo district on the coast; and from Taveta.

Evans and Garnham (1936) have described certain differences, chiefly pupal and biological, between the Kisumu and Digo forms of the species. They have also shown that the Kisumu form at least is not found in native huts in the neighbourhood of the breeding places.

Anopheles lesoni Evans

No larvae of this species were found during the visit of one of us (A. M. E.) to Kenya, but an isolated ♀ with its pelts, collected earlier in the Meru district, was examined; its characters were as follows:—

ADULT. ♀. Agreeing exactly with characteristic South African specimens, in which the third vein is pale with small dark spots towards the ends. Pharynx with hairs of post-armature ridges short, as in *lesoni* (De Meillon, 1935; Evans and Leeson, 1935).

LARVA. Agreeing with typical *lesoni* in all characters present, including branching of hair 'O'.

PUPA. Agreeing in almost all respects with South African material. Bristle C on segment VII, however, was not simple, but showed a delicate secondary branch at about its middle.

Collections of small numbers of larvae were examined in the laboratory at Nairobi and were found to agree exactly with Southern Rhodesian specimens. As in the material recorded by one of us (A. M. E., 1936), the shape of the main tergal plates was found to be remarkably constant, and the dark posterior rim (Evans and Leeson, 1935) was characteristically present.

A. lesoni can now be recorded from the following localities in Kenya: Meru (Kathura); Kibwezi; Kyale (Teita Reserve); Fort Hall (formerly present, at least).

The species is evidently absent from the coastal belt and from the Kisumu district of the Kavirondo Gulf. The Fort Hall record is interesting. It is based on nine larvae collected from a breeding place which has since been controlled; this is the greatest number of larvae of the species collected in any of the above localities. In September, 1936, a trained mosquito-boy spent a month at Fort Hall collecting and rearing larvae, especially of the *funestus* series; during this time larvae of *A. funestus* type form were found in at least eight different breeding places; but not a single larva of *A. lesoni* was discovered.

SUMMARY

Three members of the *funestus* series, *A. funestus* type form, *A. rivulorum* and *A. lesoni*, can definitely be recorded from Kenya.

In comparison with the common and widely distributed *A. funestus* type form, *A. rivulorum* and *A. lesoni* may be described as localized or rare.

It is more than probable that in most parts of the Colony, at least, *A. funestus* type form is the only one of these three species which is found in appreciable numbers in huts or houses; thus there is little, if any, doubt that records of natural infectivity in '*funestus*' in the Colony do actually apply to the type form of this species.

Many of the larvae raised from eggs at Kisumu were abnormal in having the paired platelets isolated from the main plate on some or all of segments IV-VI. This condition was extremely rare among caught larvae. Such specimens differ from larvae of *A. funestus* var. *confusus* in having the main tergal plates unusually narrow.

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EXPLANATION OF PLATE IV

Unusual breeding places of *A. funestus* type form at Malindi, described by one of the authors (Symes, 1936). (All photographs by C. B. Symes.)

Fig. 1. A well outside a mosque.

Fig. 2. One of the numerous wells for domestic use. Behind is a typical mud-and-pole house.

Fig. 3. Well outside a mosque.

Larvae of *A. funestus* were found in the wells, and also in stone or cement tanks inside the mosques and houses.



Fig. 1



Fig. 2



Fig. 3

H. R. Grubb, Ltd., Poplar Walk, Croydon



STUDIES ON THE HIGHER DIPTERA OF MEDICAL AND VETERINARY IMPORTANCE

THE BOT FLIES OF THE SUBFAMILY OESTRINAE

(Continued from Vol. 30, page 468)

BY

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(Received for publication 3 February, 1937)

In their monograph of the parasitic Diptera of Africa, Rodhain and Bequaert (1916) revised the species of bot flies of the subfamily Oestrinae, placing them in the genera *Oestrus* L., *Cephalopsis* Townsend, *Rhinoestrus* Brauer, *Kirkioestrus* Rodhain and Bequaert, and *Gedoelstia* Rodhain and Bequaert, and recognizing the following species :—

SPECIES	HOST
<i>Cephalopsis titillator</i> Clark	Camel, dromedary
<i>Oestrus ovis</i> L.	Sheep, goat
<i>Oestrus variolosus</i> Lw.	Hartebeests, <i>Bubalis major</i> and <i>B. lewel jacksoni</i> , and probably other antelopes
<i>Oestrus aureo-argentatus</i> Rod. and Beq.	Roan, <i>Hippotragus equinus</i> , and hartebeests, <i>Bubalis major</i> and <i>B. lewel jacksoni</i>
<i>Oestrus macdonaldi</i> Gedoelst (adult unknown)	Hartebeest, <i>B. lichtensteini</i>
<i>Rhinoestrus nivaraleti</i> Rod. and Beq.	Red river-hog, <i>Potamochoerus porcus</i>
<i>Rhinoestrus purpureus</i> Brauer	Equidae
<i>Rhinoestrus hippopotami</i> Grünberg	<i>Hippopotamus amphibius</i>
<i>Rhinoestrus phacochoeri</i> Rod. and Beq.	Wart-hog, <i>Phacochoerus aethiopicus</i>
<i>Gedoelstia cristata</i> Rod. and Beq.	Hartebeest, <i>B. lichtensteini</i>
<i>Gedoelstia paradoxa</i> Rod. and Beq. (<i>hässleri</i> Ged.) (adult unknown)	Hartebeest, <i>B. lewel jacksoni</i> ; topi, <i>Damiliscus korrigum jumela</i>
<i>Kirkioestrus surcoufi</i> Ged.	Hartebeests, <i>B. major</i> and <i>B. lewel jacksoni</i>
<i>Kirkioestrus blanchardi</i> Ged. (adult unknown)	Hartebeest, <i>B. lichtensteini</i>
<i>Kirkioestrus minutus</i> Rod. and Beq.	Hartebeest, <i>B. lewel jacksoni</i> ; topi, <i>Damiliscus korrigum jumela</i>
The following species have also been recorded :—	
<i>Oestrus disjunctis</i> Ged.	Roan, <i>Hippotragus equinus</i> , and bastard hartebeest
<i>Oestrus compositus</i> Ged.	Hartebeest, <i>B. lichtensteini</i>
<i>Oestrus regalis</i> Austen	Tiang, <i>Damiliscus korrigum tiang</i>
<i>Gedoelstia impolita</i> Austen	Blue wildebeest, <i>Gorgon taurinus</i>
<i>Oestrus macropi</i> Froggart	Kangaroo

It will be remembered that all these flies are parasitic in their larval stages in the nasal and accessory sinuses of their hosts. All are larviparous in habit, the ♀ depositing the first stage larvae at the entrance of the nostrils; the larvae then pass up the nasal passages into the various sinuses of the skull-bones communicating with them. In these sites they fix themselves by the aid of

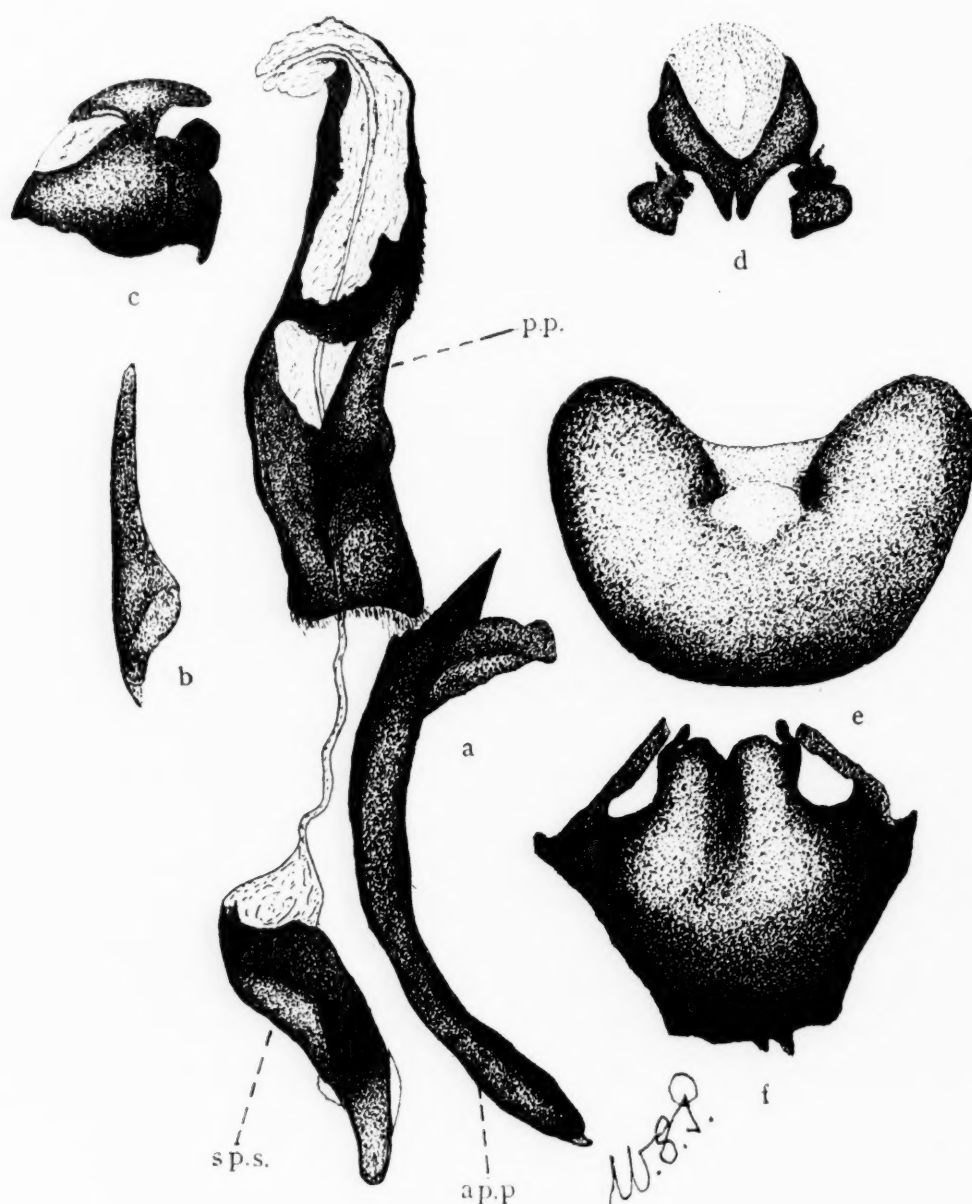


FIG. 1. *a.*—Phallosome and paramere of *Cephalopsis titillator* in side view; *b.*—Posterior paramere; *c.*—Tenth tergum, anal cercus and ninth coxite in side view; *d.*—Ventral view of anal cerci and distal segment of ninth coxite; *e.*—Fifth sternum; *f.*—Ninth tergo-sternum.

their strong oral hooks to the mucus membrane. The larvae cause considerable irritation, resulting in excessive nasal discharge and the condition known as 'staggers.' The only way to obtain the adult flies is to hatch them out from larvae, and, as Roubaud has pointed out, this can be done by opening the skull and breaking off the horns at the base, when the larvae can be collected from

the cavities. Very often the larvae will crawl out from the nostrils of a dead animal. Only those larvae which have dark brown or black bands on the body should be collected, as these are nearing maturity; entirely white larvae should not be collected, as they will never pupate and are best preserved in spirit. The nearly mature dark larvae should be placed in a small tin, in which there is a layer of dry earth several inches deep, and the lid should be perforated. The larvae will bury themselves in the earth and pupate. They should be left undisturbed, and the adults will hatch out in from 4 to 6 weeks. These mature larvae should never in the first instance be placed in test-tubes several of them together. When the adults have hatched, they should be allowed to harden for at least 24 hours, and should then be pinned with their puparia. I sincerely hope that medical officers and others who have opportunities of collecting these interesting larvae will do so, and will hatch out the adults.

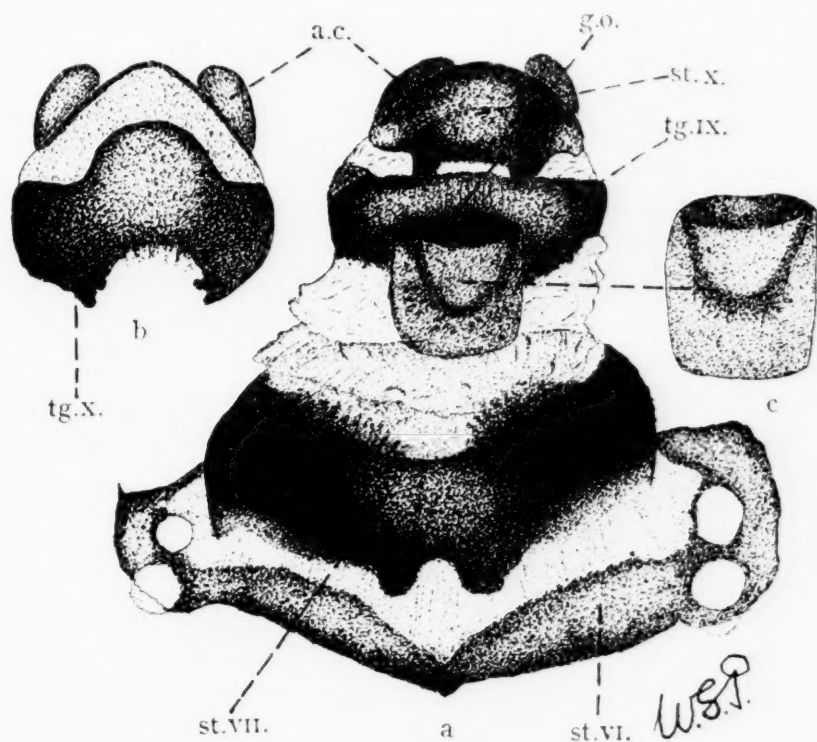


FIG. 2. *a*.—Ventral view of terminalia of *Cephalopsis titillator*; *a.c.*—Anal cercus; *g.o.*—Genital opening; *st.vi.*, *st.vii.*, *st.x.*—Sixth, seventh, tenth sternum; *tg.ix.*, *tg.x.*—Ninth, tenth tergum; *b*.—Dorsal view of end of larvipositor; *tg.x.*—Tenth tergum; *c*.—Ninth sternum.

In the present paper I propose to note and to illustrate the salient diagnostic characters of the terminalia of *Cephalopsis titillator*, *Oestrus ovis*, *O. variolosus* and *O. aureo-argentatus*. Unfortunately, I have not had an opportunity of studying the terminalia of any species of *Rhinoestrus*, *Geddoelstia* or *Kirkioestrus*. In addition, I am able to note and illustrate the terminalia of *Cephenemyia auribarbis* and *C. stimulator*; for specimens of the latter I am indebted to Dr. Sczilady. As the segmentation of the abdomen is similar to that of the other parasitic Muscidae, I do not propose to describe it.

Cephalopsis titillator Clark. The nasal bot of the camel and dromedary. MALE TERMINALIA. Fig. 1. The fifth sternum (fig. 1, *e*) is wide, the lateral lobes broadly rounded; terga 6 and 7 are fused, and tergum 10 is short, wide, and with a deep incision. The ninth tergo-sternum (fig. 1, *f*) is raised in the middle line, and the posterior processes are long, narrow and directed inwards. The proximal segment of the ninth coxite (fig. 1, *c*) is a short wide plate, articulating in the usual way against the posterior processes of the ninth tergo-sternum; the distal segment (fig. 1, *c, d*) is small, the end broadly rounded and directed inwards towards its fellow. The anal cerci (fig. 1, *c, d*) are small, their anterior free ends short and bluntly pointed; they are fused posteriorly and extend backwards on each side of the anal opening. The proximal part of the body of the phallosome (fig. 1, *a*) is long and wide, and is joined to the distal part by chitin ventrally and by membrane dorsally. The two chitinous parts of the distal end are joined proximally by a bar, which is continuous with the dorsal part of the distal end; the distal end is membranous and carries the end of the ejaculatory duct. The anterior part of the paramere is wanting; the posterior part (fig. 1, *a, b*) is a long, narrow, bluntly pointed, finger-like plate bearing the usual sensory hairs.

FEMALE TERMINALIA. Fig. 2. The larvipositor is elongated and wide. Tergum and sternum 6 are joined together to form a chitinous ring, the sternum bearing the sixth and seventh spiracles at the sides. Tergum and sternum 7 are similarly fused to form a chitinous ring, sternum 7 being the wider of the two. Sternum 9 is a small rectangular plate lying in the middle line; tergum 9 is a wide plate, bent round on the ventral side. Sternum 10 is a large, somewhat triangular plate, with two antero-lateral projections supporting the genital opening. Tergum 10 is a large plate rounded anteriorly in the middle line and emarginated posteriorly. The anal cerci are small elongated rounded plates.

Oestrus ovis L. The nasal bot of the sheep. MALE TERMINALIA. Fig. 3. Sternum 5 (fig. 3, *e*) is narrower and has not such prominent lobes as that of *C. titillator*. The ninth tergo-sternum (fig. 3, *f*) is structurally similar to that of *C. titillator*, but there is a large membranous area in the middle, and the raised middle portion consists of stout, bent, rod-like processes; the lateral processes are long and bent inwards. The ninth coxite (fig. 3, *c, d*) is somewhat similar to that of *C. titillator*, the distal segment is larger and is bluntly pointed at the end; the proximal segment is a short, rather narrow rod. The anal cerci (fig. 3, *c, d*) are larger and longer than those of *C. titillator*, the free anterior end is longer and bluntly pointed, the remaining portion is fused. The lateral view of the anal cerci and ninth coxite is shown in fig. 3, *c*. The phallosome (fig. 3, *a*) is structurally similar to that of *C. titillator*, but is very short. The proximal part ends in a short, dorsally bent, posterior process; the distal part is particularly short and wide, but is in all other respects similar to that of *C. titillator*, the two chitinous parts being joined by a long wide bar of chitin; the dorsal part has many fine teeth. As in *C. titillator*, the anterior part of the

paramere is wanting; the posterior part (fig. 3, *a*, *b*) consists of a short, wide, bent plate, with the usual sensory hairs. The apodeme of the phallosome is very long and wide.

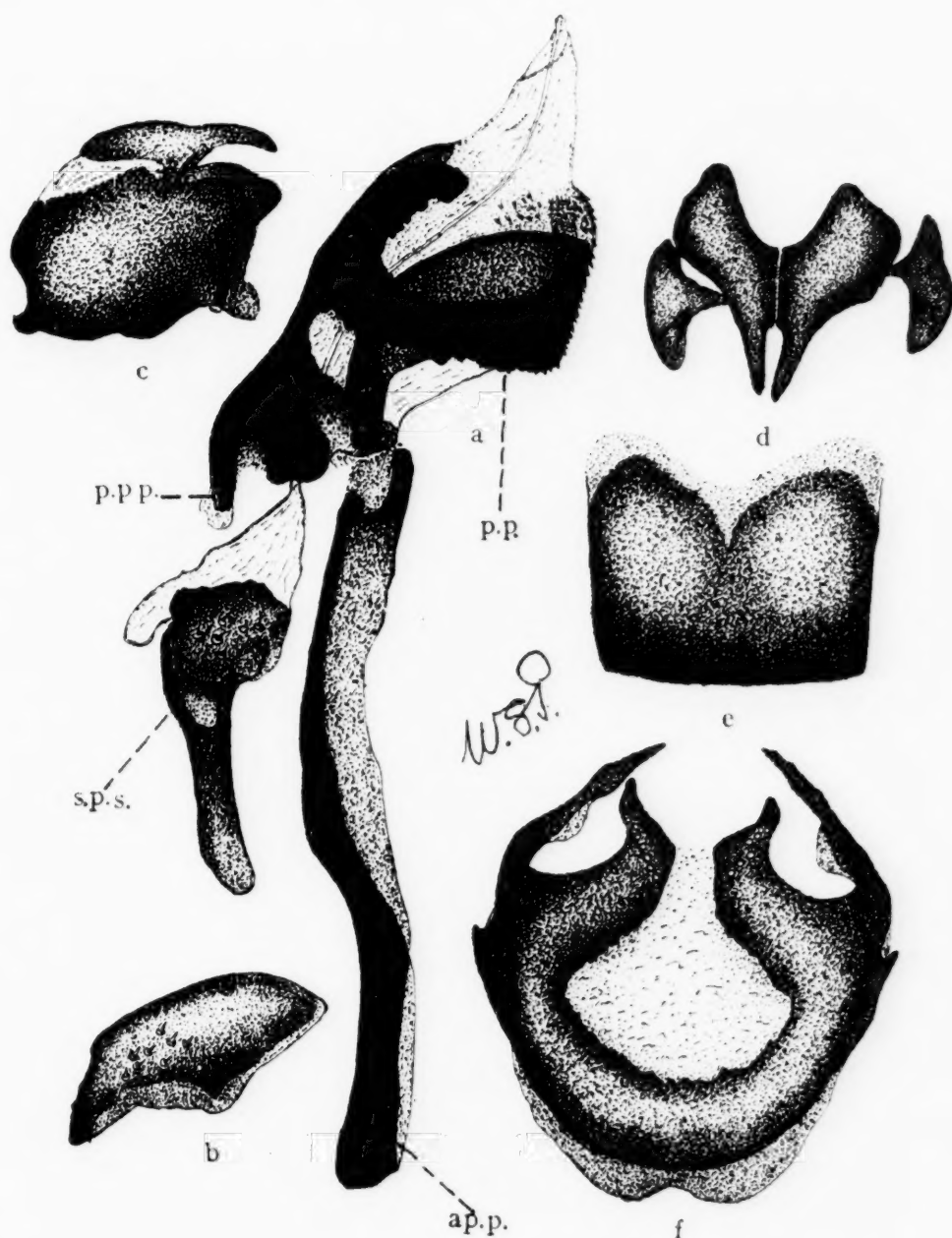


FIG. 3. *a*.—Phallosome and paramere of *Oestrus ovis* in side view; *b*.—Posterior paramere; *c*.—Tenth tergum, anal cercus and distal segment of ninth coxite in side view; *d*.—Ventral view of anal cerci and distal segments of ninth coxite; *e*.—Fifth sternum; *f*.—Ninth tergo-sternum.

FEMALE TERMINALIA. Fig. 4. The larvipositor is long and relatively wide. Sterna 3, 4 and 5 each consist of two parts, the intervening area more or less membranous. Tergum and sternum 6 are well developed. Tergum 7 is apparently wanting. Sternum 7 is Y-shaped and pointed in the middle line posteriorly and projects under sternum 6. Tergum 9 (fig. 4, *b*) is a long rounded

plate, narrow in the middle; sternum 9 is a wide triangular-shaped plate. Sternum 10 (fig. 4, *b*) is also triangular in shape, and is divided into two parts by a central membranous area. Tergum 10 (fig. 4, *e*) is a long narrow plate; the anal cerci are small rounded plates.

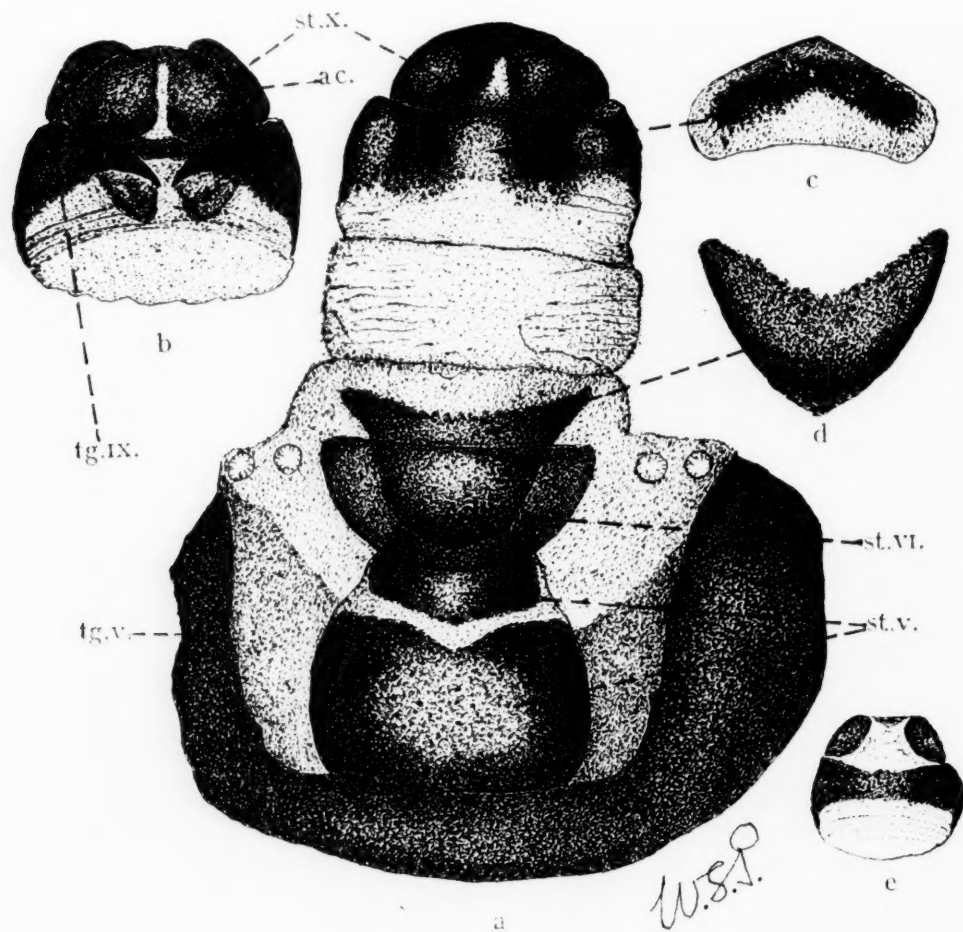


FIG. 4. *a*.—Ventral view of terminalia of ♀ *O. ovis*; lettering as in fig. 2, *a*; *b*.—Ventral view of end of larvipositor; *c*.—Ninth sternum; *d*.—Seventh sternum; *e*.—Dorsal view of end of larvipositor, showing ninth tergum and anal cerci.

Oestrus variolosus Loew. MALE TERMINALIA. Fig. 5. The fifth sternum (fig. 5, *f*) is a rather narrow, short, wide plate with rounded lobes, and is very similar to the fifth sternum of *O. ovis*. The ninth tergo-sternum (fig. 5, *b*) is a large wide plate, very similar to that of *O. ovis*. The proximal segment of the ninth coxite is a short wide plate; the distal segment (fig. 5, *d, e*) is short, rounded at the free end and turned inwards towards its fellow. The anal cerci (fig. 5, *d, e*) are long, the distal free end of each is bluntly pointed, and posteriorly each cercus is short and broadly rounded; the anal cercus is seen in side view in fig. 5, *e*. The proximal part of the phallosome (fig. 5, *a*) is short and wide, the posterior process long and deeply cleft, and projects backwards in the shape of a hump; the distal part of the phallosome is very similar to that of *O. ovis* and (especially) to that of *C. titillator*, the difference being that basally the dorsal

and ventral chitinous parts are joined to form a short tube ; the short dorsal chitinous part has numerous small teeth along its edge. The posterior part of the paramere (fig. 5, *a*, *c*) is structurally similar to that of *O. ovis* ; as in the other species, the anterior part is wanting. I have not had an opportunity of studying the female terminalia of this species.

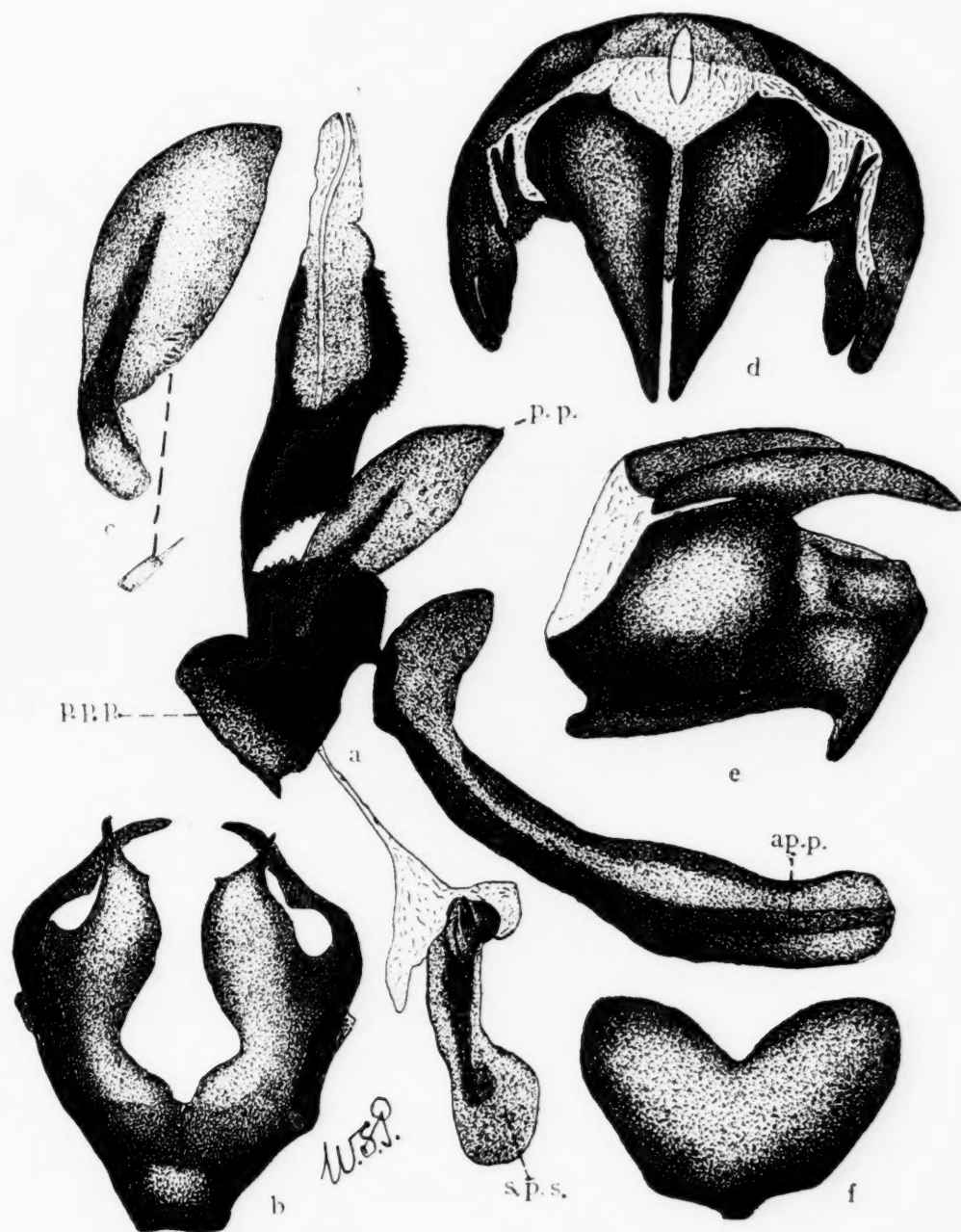


FIG. 5. *a*.—Phallosome and paramere of *O. variolosus* in side view ; *b*.—Ninth tergo-sternum ; *c*.—Posterior paramere ; *d*.—Ventral view of anal cerci and distal segments of ninth coxite ; *e*.—Tenth tergum, anal cercus and ninth coxite in side view ; *f*.—Fifth sternum.

Oestrus aureo-argentatus Rod. and Beq. I have not been able to study the male terminalia of this species, as no specimen is available. FEMALE TERMINALIA. Fig. 6. The larvipositor of this species is very similar to that of *O. ovis*. The differences are to be noted in the size and shape of the sternites; they need not be described, but can be noted on comparing fig. 4 with fig. 6.

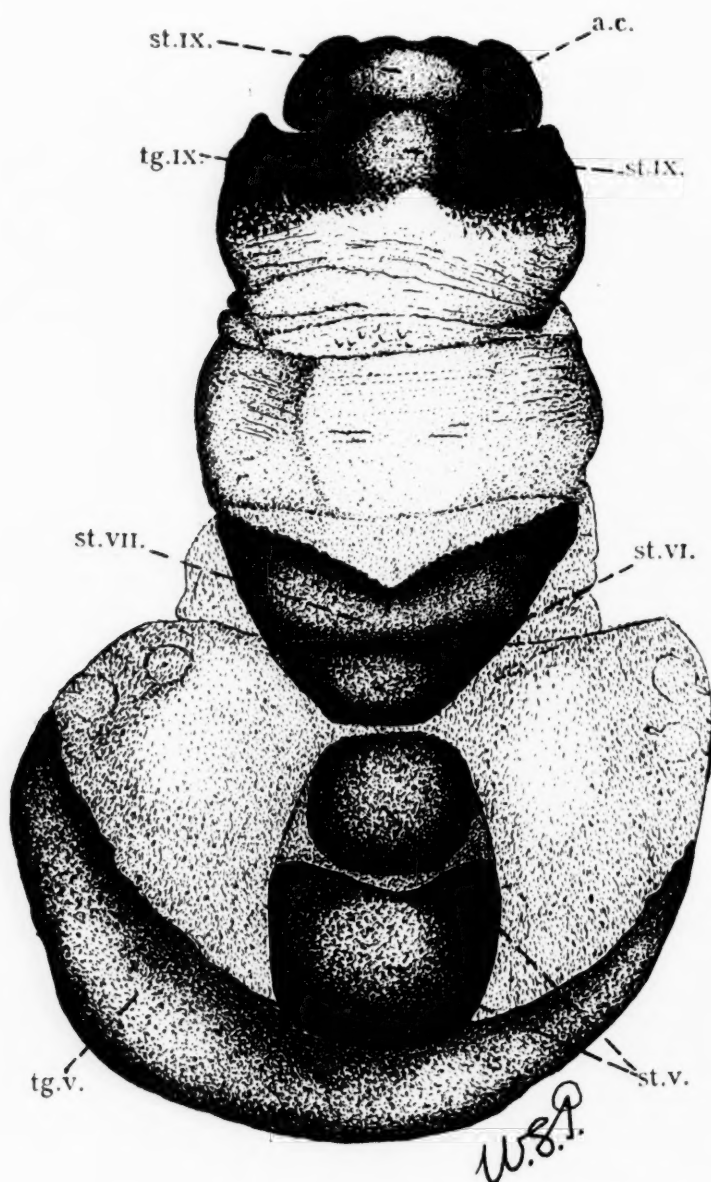


FIG. 6. Ventral view of ♀ terminalia of *O. aureo-argentatus*; lettering as in fig. 2, a.

Cephenemyia auribarbis Meig. MALE TERMINALIA. Fig. 7. The fifth sternum (fig. 7, f) is narrow, with long rounded lateral lobes. The proximal segment of the ninth coxite (fig. 7, d) is a short broad plate; the distal segment is a long, bluntly pointed plate, directed inwards towards its fellow; the lateral view is shown in fig. 7, b. The anal cerci (fig. 7, b, d) are long, with narrow, bluntly pointed, free ends. The posterior part of each cercus projects backwards

on each side of the anal opening ; the lateral view of the anal cercus is shown in fig. 7, *b*. The phallosome is relatively short, the proximal part of the body wide, the posterior process forming a long deeply cleft plate ; the distal part of the phallosome is like that of *C. titillator*, *O. ovis* and *O. variolosus* ; the dorsal

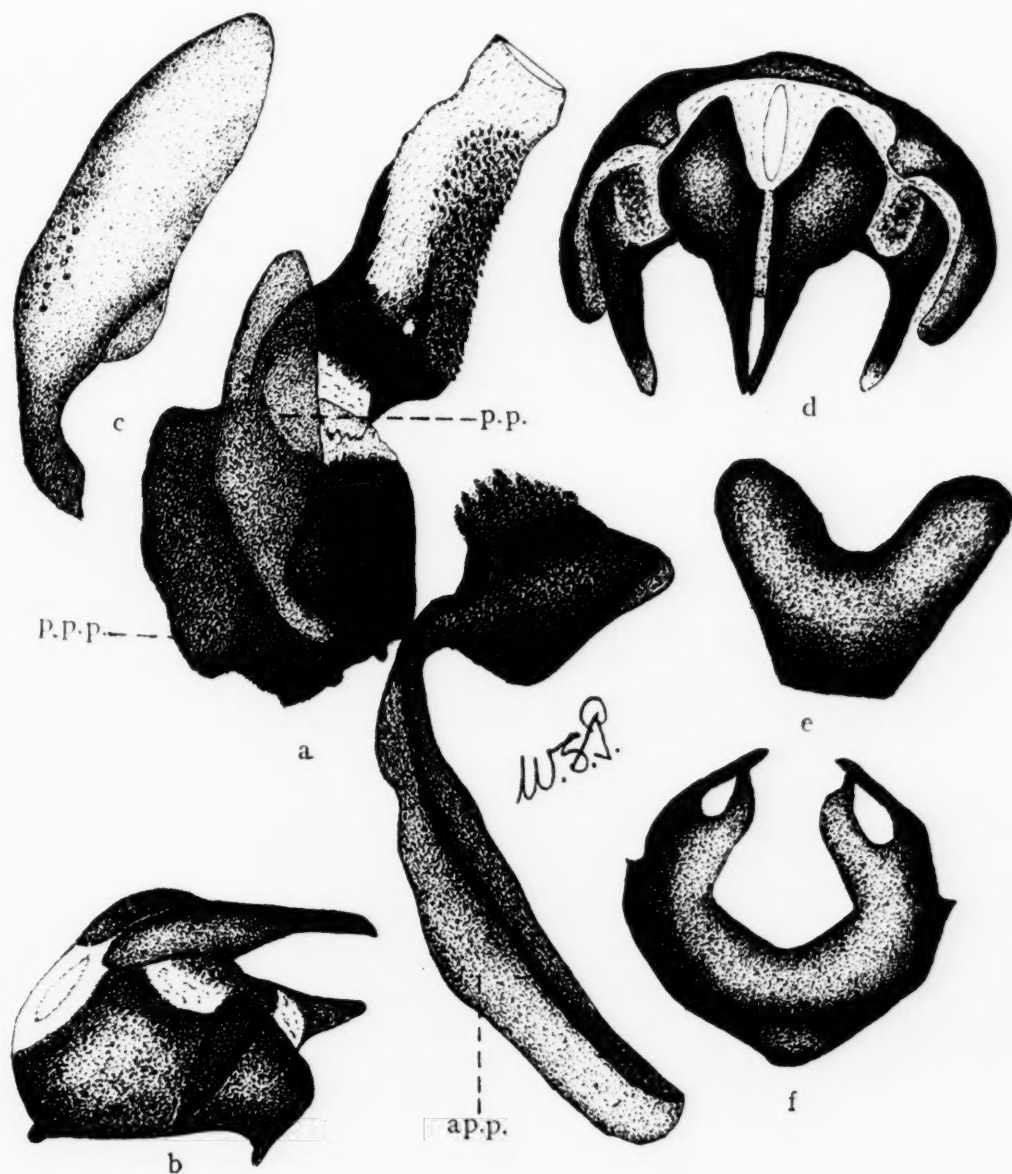


FIG. 7. *a*.—Phallosome and paramere of *Cephemyia auribarbis* in side view ; *b*.—Tenth tergum, anal cercus and ninth coxite in side view ; *c*.—Posterior paramere ; *d*.—Ventral view of anal cercus and distal segment of ninth coxite ; *e*.—Fifth sternum ; *f*.—Ninth tergo-sternum.

chitinous part is thickly beset by small pointed spines, and the bar joining the two parts is wide ; the distal membranous part is wide, the opening of the ejaculatory duct large. The paramere (fig. 7, *a*, *c*) is a broad bluntly pointed plate.

FEMALE TERMINALIA. Fig. 8. The larvipositor is elongated, and all the sterna and terga are present and well developed. There is a stout dagger-shaped plate apparently attached to sternum 6. In all other respects the larvipositor is very similar to that of the other species noted above.

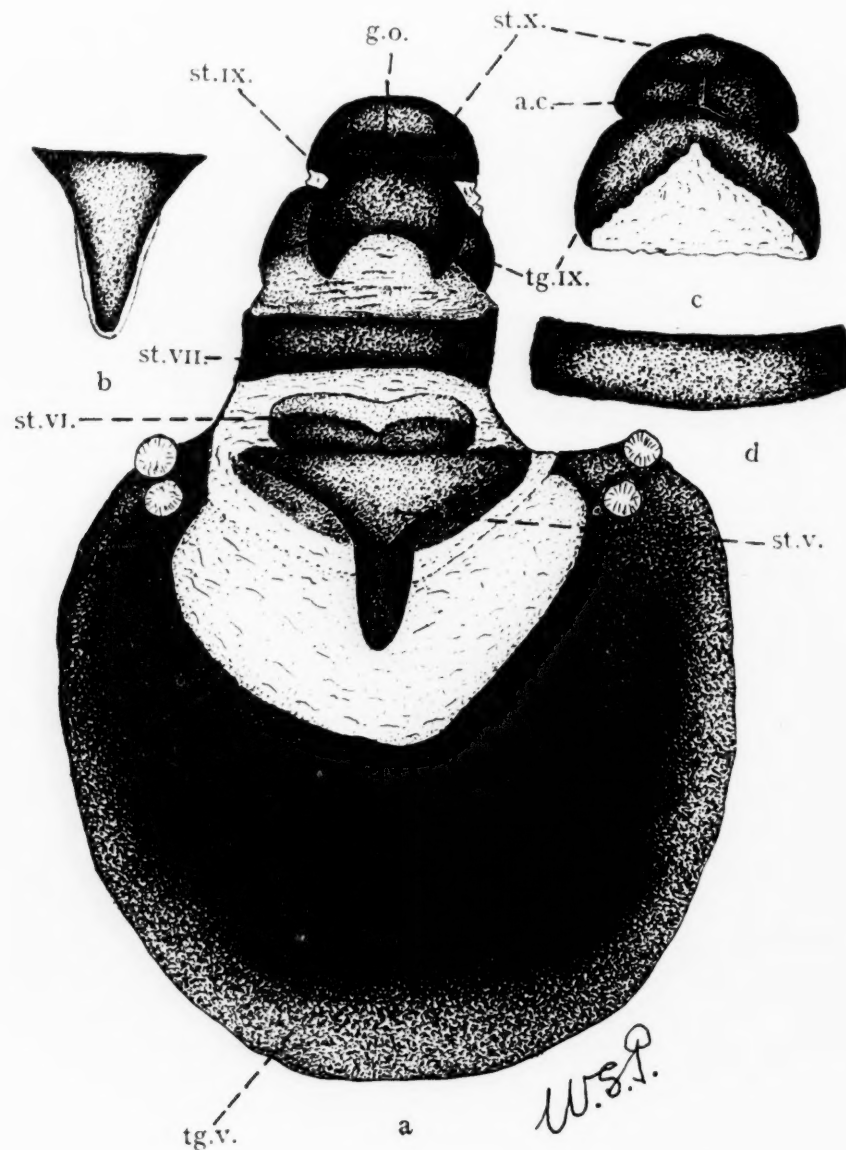


FIG. 8. *a*.—Ventral view of terminalia of ♀ *C. auribarbis*; *st.v.*—Fifth sternum; *tg.v.*, *tg.ix.*—Fifth, ninth tergum; other lettering as in fig. 2, *a*; *b*.—Dagger-like chitinous process attached to sixth sternum; *c*.—Dorsal view of end of larvipositor; *d*.—Sixth tergum.

Cephenemyia stimulator Clark. MALE TERMINALIA. Fig. 9. The fifth sternum (fig. 9, *f*) is wide, with rounded lateral lobes, and is structurally similar to that of the species of *Oestrus*. The ninth tergo-sternum (fig. 9, *e*) is structurally like that of the *O. ovis*, etc. The proximal segment of the ninth coxite (fig. 9, *b*) is a long broad plate; the distal segment (fig. 9, *b*) is short and narrowly rounded at the end. The anal cerci (fig. 9, *b, d*) are long, the distal free ends wide and

bluntly pointed. The phallosome (fig. 9, *a*) is very similar to that of *O. variolosus* and *C. auribarbis*, and like the latter is heavily armed with fine spines. The paramere (fig. 9, *a*, *c*) is a long, wide, rounded plate. I have not had an opportunity of examining the ♀ terminalia of this species.

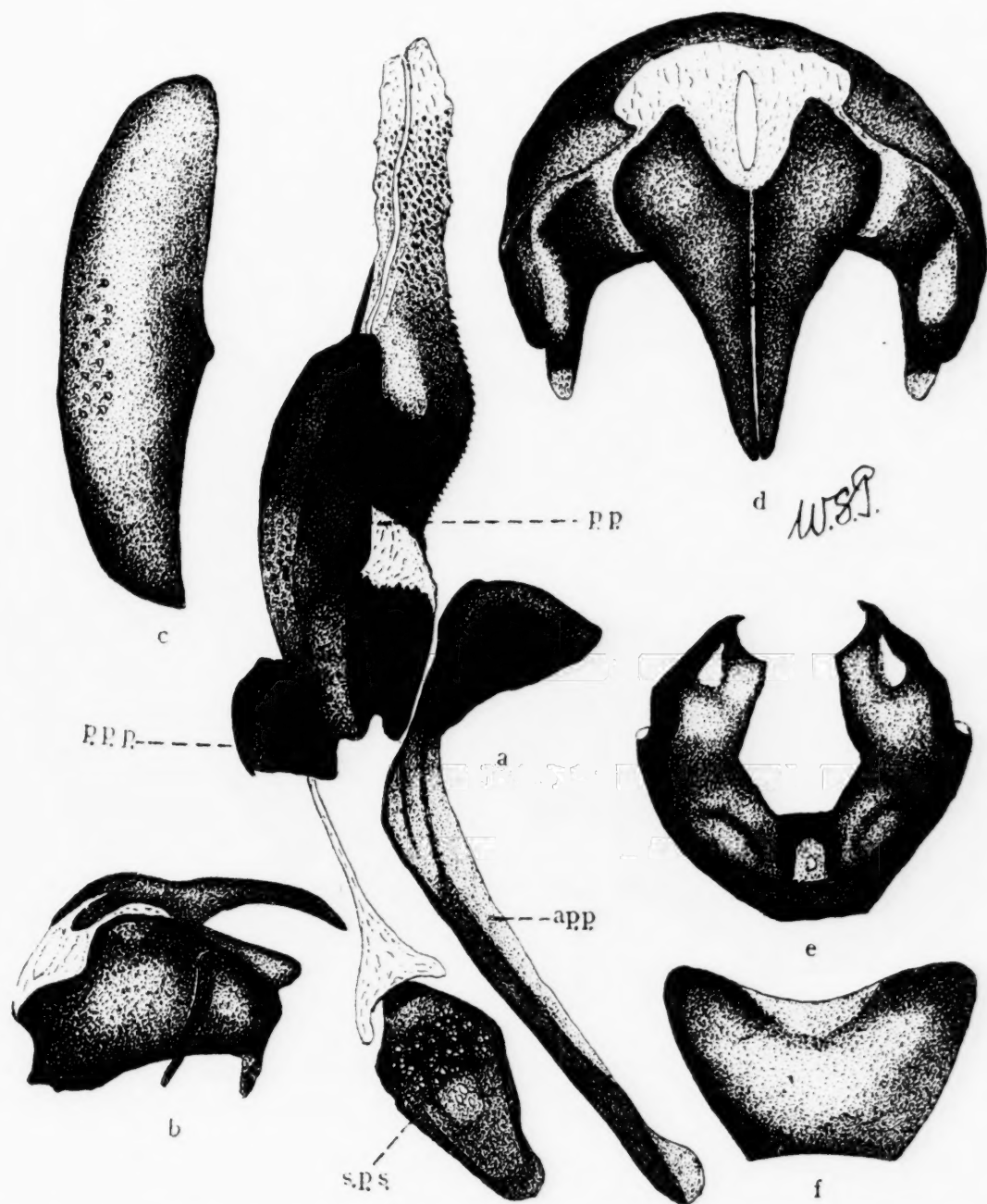


FIG. 9. *a*.—Phallosome and paramere of *C. stimulator* in side view; *b*.—Tenth tergum, anal cercus and ninth coxites in side view; *c*.—Posterior paramere; *d*.—Ventral view of anal cerci and distal segment of ninth coxites; *e*.—Ninth tergo-sternum; *f*.—Fifth sternum.

NOTES. The study of the terminalia of *C. titillator*, *O. ovis*, *O. variolosus* and *O. aureo-argentatus* leaves no doubt that we have here a natural group of closely related species. The structure of the fifth sternum, the ninth tergo-sternum, the ninth coxites and anal cerci, the phallosome and paramere, and the

larvipositor are fundamentally similar in all. The length of the larvipositor is of interest, and shows that in viviparous Diptera this structure may be relatively long; its length is in marked contrast to that of the larvipositor of *Sarcophaga* and *Glossina*. It seems to me that the long larvipositor in this group is necessary in order that the larvae may be deposited well within the opening of the nostril. I have observed the ♀ *C. titillator* sitting on the end of the nose of a dromedary when larvipositing, and have no doubt that the long larvipositor enables it to bend it round and deposit the larvae well within the opening of the nostril. The

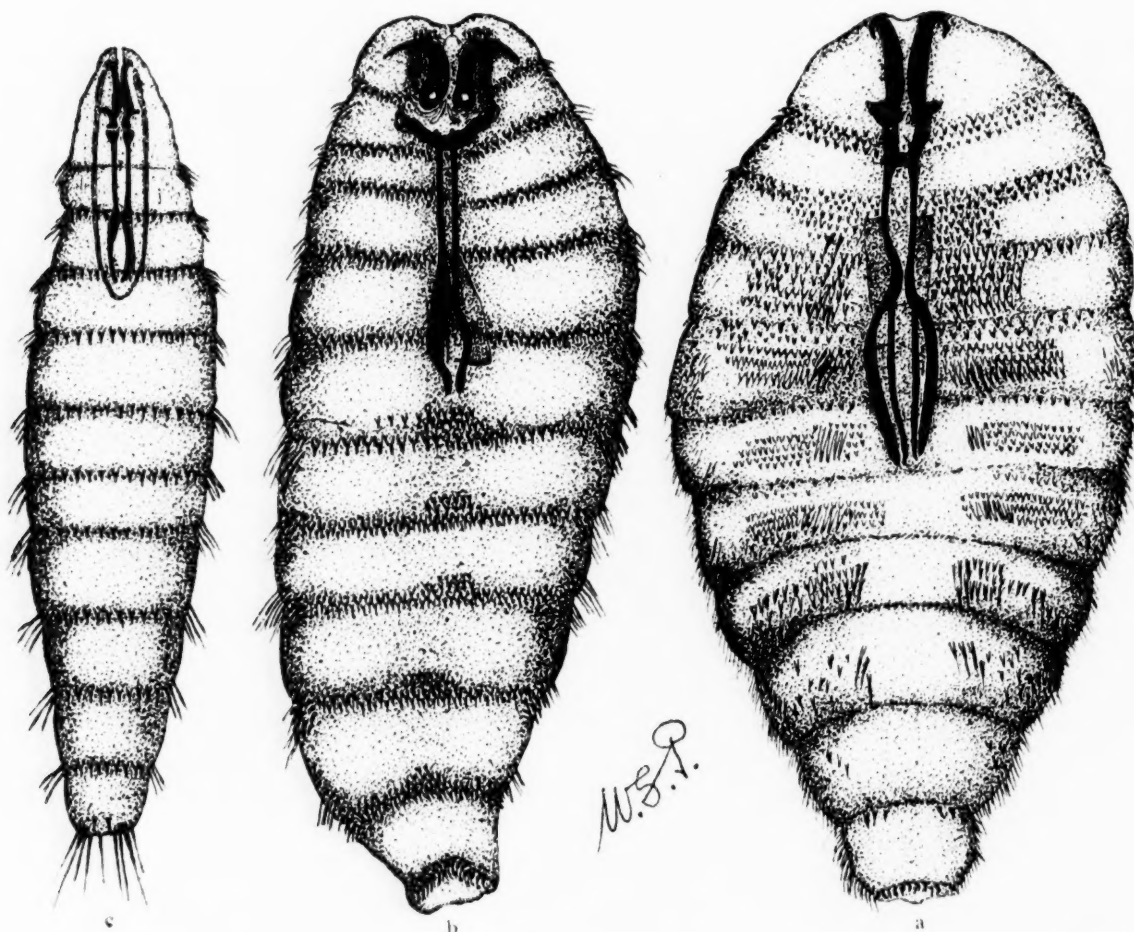


FIG. 10. a.—Ventral view of first stage larva of *Cephenemyia auribarbis*; b.—Same of *Oestrus ovis*; c.—Same of *Cephalopsis titillator*.

♂ terminalia are structurally adapted to operate with such a long larvipositor, as in all the ninth coxites are well developed and function as lateral claspers, the proximal segment acting as a lever.

The structure of the terminalia of two species of *Cephenemyia* is also of peculiar interest. It will be remembered that the flies of this genus deposit their first stage larvae in the nostrils of deer. The adults are externally quite unlike the species of the Oestrinae, being large hairy flies. The study of the terminalia of the two species recorded here demonstrates very clearly their close relationship with the Oestrinae. The reader should compare in particular the structure of

the phallosome, which is strikingly like that of *O. variolosus*, only differing in having more spines. In *Cephenemyia* there is also only one part of the paramere. Although I have only had the opportunity of examining the ♀ terminalia of *auribarbis* (the females are very rare in collections), it is structurally similar to those of the Oestrinae.

Lastly, I would draw attention to the structural similarity of the first stage larva of *Cephalopsis*, *Oestrus* and *Cephenemyia* (fig. 10). I have always maintained that it is the first stage larva of the higher Diptera which usually exhibits common group characters much better than the mature larva. The first stage larva of the three species illustrated are somewhat flattened dorso-ventrally, and are armed with rows of fine pointed appressed spines, and also with long marginal hair-like ones. Taking all these characters into account, it is, I think, clear that the *Cephenemyia* is closely related to the Oestrinae, and I therefore include it as a genus in the subfamily Oestrinae, which is its correct systematic position. In my next paper in this series I shall describe the terminalia of two species of *Cobboldia*.

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(To be continued)

STUDIES ON THE HIGHER DIPTERA OF MEDICAL AND VETERINARY IMPORTANCE

A REVISION OF THE SPECIES OF THE GENUS *MUSCA*,
BASED ON A COMPARATIVE STUDY OF THE MALE
TERMINALIA

IV.—A PRACTICAL GUIDE TO THE ORIENTAL SPECIES

(Continued from Vol. 30, page 490)

BY

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(Received for publication 3 February, 1937)

Since the publication of my earlier revision (1924) of the Oriental species, I have had the opportunity of studying some fresh material. I am indebted to Dr. Green, for a large collection from Malay ; to Mr. Carter, for the opportunity of studying a small collection from Ceylon ; to the Imperial Entomologist, the Government of India, for several collections ; to Mr. Colbran J. Wainwright, for a collection from Cachar, Assam ; and I have also been able to examine a collection made by Mr. Ch'i Ho in the Island of Hainan. I wish to thank these gentlemen for the trouble they have taken in sending me these collections. There are, however, three species whose male terminalia I have not been able to examine, viz., *Musca lucens* Villeneuve, from Ceylon, *M. jacobsoni* Malloch and *M. tibiseta* Malloch, from Sumatra ; although I have been able to examine specimens of *lucens*, I have not seen any specimens of the two species from Sumatra.

As in my previous papers, I shall not redescribe those species which also occur in either the Palaearctic or the Ethiopian regions. Notes on the adults, and illustrations of the ♂ terminalia and of the posterior spiracles of the third stage larva, will be found in my previous papers ; that published in 1932 I shall refer to as Part 1 (Pt. 1), that on the Palaearctic species (1933*b*) as Part 2 (Pt. 2), and that on the Ethiopian species (1936) as Part 3 (Pt. 3). As in the case of the species of the two other regions referred to, those of the Oriental region fall into the three groups, *domestica*, *sorbens* and *lusoria*.

DOMESTICA GROUP

***Musca domestica* L.** As far as I know, the true *domestica* does not occur in India. Doubtless it is introduced into ports from Europe by ships ; and, though I have seen doubtful specimens, I have not seen a series, which one would expect had it established itself in the country. It undoubtedly enters north India from the Palaearctic region. For notes on the adults and an illustration of the posterior spiracles of the third larva, see Pt. 2 ; for illustrations of the ♂ terminalia, see Pt. 1.

Musca vicina Macquart. This species is the common bazaar- and house-fly of India. For notes on the adults, illustrations of the ♂ terminalia and the posterior spiracles of the third larva, see Pt. 2.

Musca nebulo Fabricius. Ent. Syst., IV, 321, 35, 1794.

SYNONYMS: *determinata* Walk. nec Patton; ? *multispina* Awati.

MALE. HEAD. Vertex narrow, about one-seventh to one-eighth width of an eye; lower part of vertex and cheeks silvery. MESONOTUM. Ground-colour

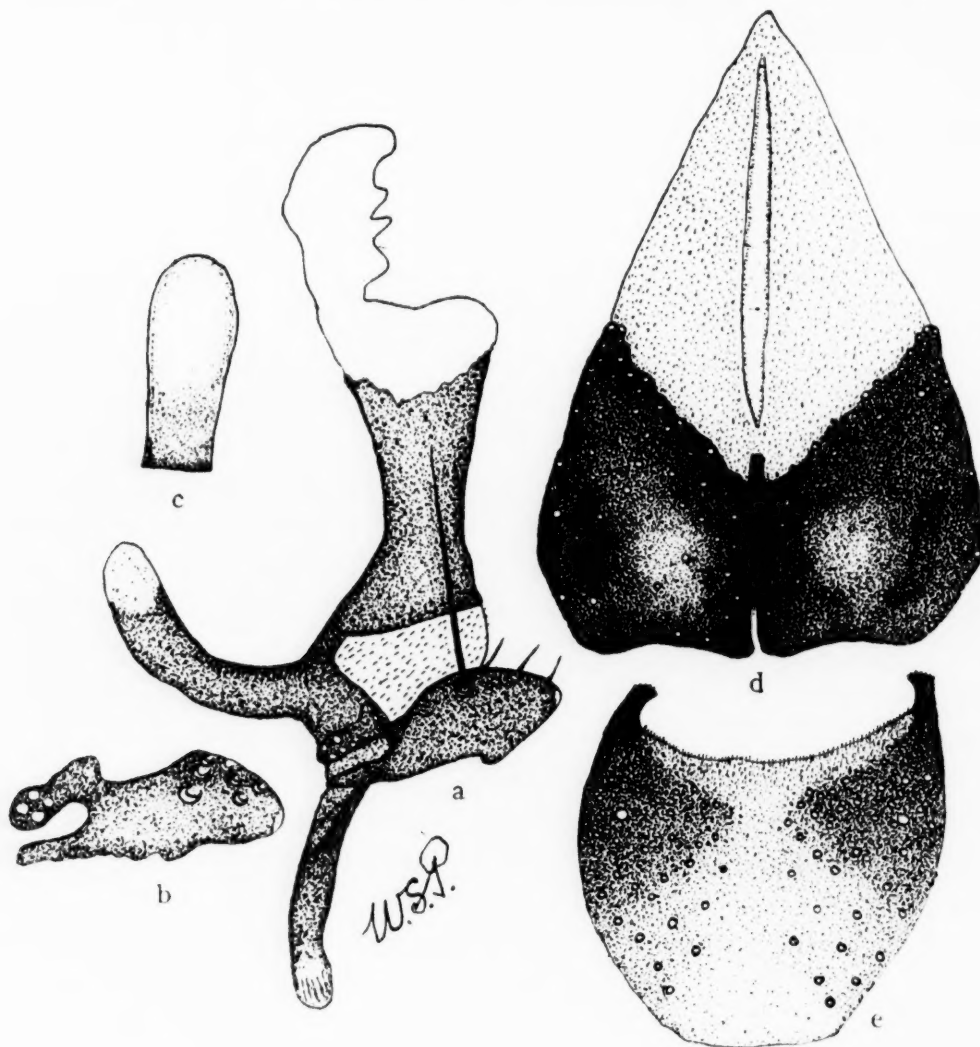


FIG. 1. *a.*—Phallosome and one paramere of *nebulo*; *b.*—Right paramere, showing seven bristles on anterior part; *c.*—Dorsal view of posterior process of phallosome; *d.*—Anal cerci; *e.*—Fifth sternum.

grey, with four black stripes. ABDOMEN. Orange; terga 1 and 2 light to dark orange, with a broad black median stripe, and about half anterior border black; tergum 3 orange, with a broad black median stripe edged on each side with a silvery stripe and with silvery marginal patches; tergum 4 very similar, but silvery markings more extensive; tergum 5 with a faint incomplete median dark stripe, the remainder silvery. Terga 3 to 5 commonly appear silvery when seen with head towards observer.

MALE TERMINALIA. Fig. 1. ANAL CERCI. Free margins with shallow emargination, outer ends rounded, and inner not forming prominent nipples. PHALLOSOME. Chitinous part long and with a waist; posterior process long and slightly expanded at end. PARAMERE. Anterior part long and deep, with one long and usually three shorter bristles; posterior part small and slightly raised. FIFTH STERNUM. Long and broad; posterior processes short, broad and bent inwards.

FEMALE. HEAD. Vertex about three-quarters (to almost) width of an eye; cheeks silvery; vertical stripe brown and wide. MESONOTUM. As in ♂. ABDOMEN. Light orange with silvery markings; terga 1 and 2 light orange, the middle of anterior border dark brown, without complete median dark stripe,

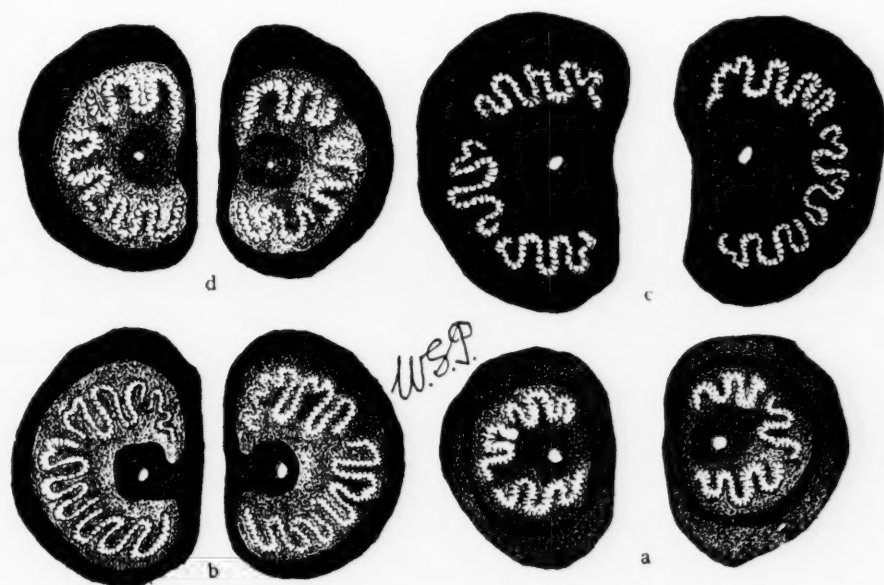


FIG. 2. *a.*—Posterior spiracles of third larva of *nebulo*; *b.*—Same of third larva of *yerburyi*; *c.*—Same of third larva of *planiceps*; *d.*—Same of third larva of *spinohumera*.

but with a dark spot at middle of posterior border; tergum 3 orange, with silvery markings, a black median stripe bordered with silvery stripes, and margins silvery; tergum 4 very similar, but median dark stripe narrower; tergum 5 mainly silvery, with some dark patches.

EARLY STAGES. Oviparous, eggs laid mainly in horse-manure, but also in human faeces. THIRD LARVA. Anterior spiracles with 5–7 finger-like processes. POSTERIOR SPIRACULAR PLATES. Fig. 2, *a.* Rounded, well separated; breathing slits rather short, wide and not much convoluted.

NOTES. This species is widely distributed in the Oriental region. In India it is a common bazaar-fly, and is found in most of the hill stations. I am still doubtful if the species described as *nebulo* from Egypt (Pt. 2) is the true *nebulo* F.

Musca planiceps Wiedemann. Auser. Zwei. Insekt., II, 414, 51, 1830.

SYNONYMS: *cingalaieina* Bigot; *pollinosa* Stein; *indica* Awati.

MALE. HEAD. Vertex very narrow, eyes approximated and bare. MESONOTUM. Four narrow black stripes; R_{4+5} with a row of small bristles on ventral surface extending beyond the radio-medial cross-vein. ABDOMEN. Pollinose yellow; terga 1 and 2 yellow, the anterior border black, and a narrow brown median stripe often incomplete; tergum 3 yellow, with a narrow brown median lunule on anterior and posterior borders sometimes joined by a brown stripe, triangular admedian stripes and silvery marginal spots; tergum 4 yellow, with a dark median posterior spot, which may be prolonged anteriorly but as a rule does not form a complete stripe, edged with silvery patches, and a narrow dark posterior band; tergum 5 mainly silvery, with suggestions of a narrow incomplete median brown stripe. The ♂ terminalia are noted and illustrated in Pt. 1.

FEMALE. HEAD. Vertex wide, almost half width of an eye. MESONOTUM. As in ♂; R_{4+5} also with bristles, as in ♂. ABDOMEN. Mainly silvery; terga 1 and 2 yellow to orange, the anterior border in the middle line black, extending posteriorly to form a narrow brown median stripe; tergum 3 silvery (orange if rubbed), with a brown median spot on posterior border, which may extend anteriorly to form a complete (or incomplete) median stripe, remainder of tergum mainly silvery; posterior margin nearly always with a narrow posterior dark band; tergum 4 similar, but with a narrower dark-brown to black stripe and a rather broad dark posterior band; tergum 5 mainly yellowish-white, with a suggestion of a median dark stripe.

EARLY STAGES. Larviparous, early third stage larva deposited one at a time in fresh cow-dung. THIRD LARVA. Anterior spiracles with 10–12 finger-like processes. POSTERIOR SPIRACULAR PLATES. Fig. 2, c. Large, peritreme wide, plates rather close, breathing slits wide and not much convoluted.

NOTES. *M. planiceps* is a widely distributed blood-sucking species; it is mainly found on cattle and on and about patches of cow-dung. It may be readily missed unless searched for in these places. The proboscis has been noted and illustrated in another paper (1933a).

Musca planiceps var. **formosana** Malloch. Mr. Ch'i Ho, who is at present working in this Department, has compared his specimens (some 46 in number), collected in the Island of Hainan, with those collected by me in India, and has noted the following differences. The Hainan specimens are on the whole more robust than the Indian ones, and in the majority there are no small bristles on the ventral side of vein R_{4+5} extending beyond the radio-medial cross-vein; in several specimens the bristles are, however, wanting. In the Indian specimens, on the other hand, again except for a few specimens, these bristles are always present, extending well beyond the radio-medial cross-vein. I have examined the ♂ terminalia of several specimens and note the following differences:—The posterior process of the phallosome of the Hainan specimens is widely expanded hood-like, and is not forked; in the Indian *planiceps*, on the other

hand, the posterior process is long, narrow and forked at the end. The chitinous part of the phallosome of the Hainan specimens is slightly longer, and the posterior part of the paramere is slightly more raised. Assuming that the Hainan specimens are identical with those from the neighbouring Island of Formosa, I propose, in view of these differences, to make it a variety of *planiceps*. It will be remembered that Malloch (1925) considered it a distinct species. I consider, however, that it would be unwise to regard it as such, until specimens are examined from the mainland of China and from other parts between the extremes of its distribution.

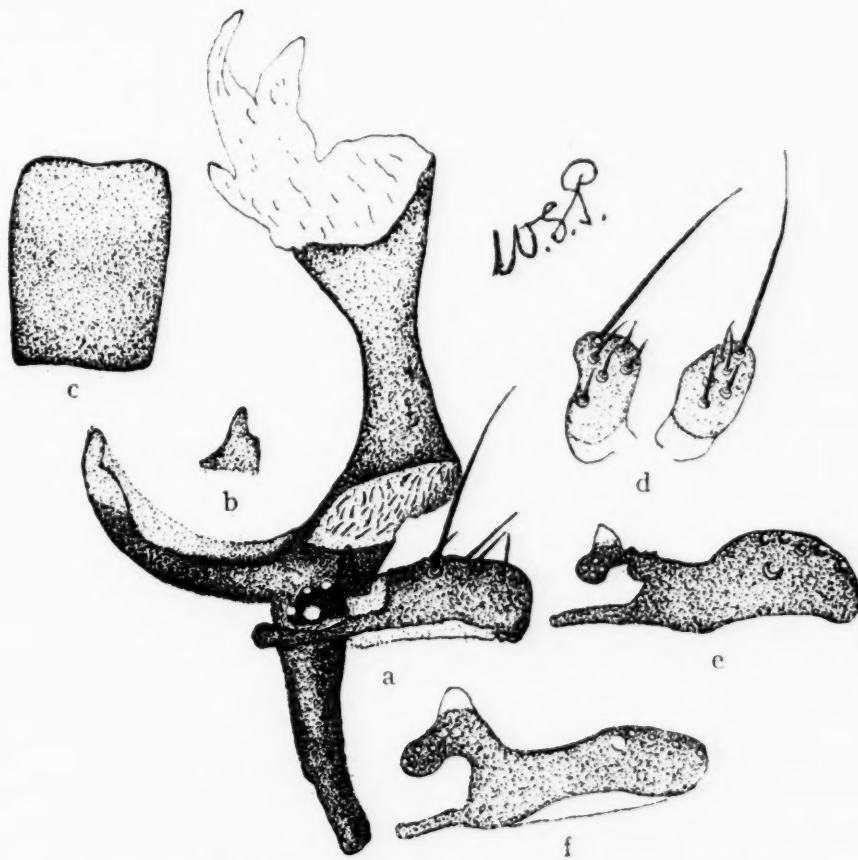


FIG. 3. *a.*—Phallosome and one paramere of *yerburyi*; *b.*—Posterior part of paramere; *c.*—Dorsal view of posterior process of phallosome; *d.*—Dorsal view of anterior parts of parameres, showing bristles; *e.*—Left paramere; *f.*—Right paramere.

***Musca yerburyi* Patton.** Philipp. Jl. Sci., XXIII, 329, 1923.

SYNONYM: *incerta* Patton nec Walker.

MALE. HEAD. Vertex very narrow, eyes approximated; cheeks silvery. **MESONOTUM.** Four broad black stripes on a dark bluish ground. **ABDOMEN.** Orange; terga 1 and 2 yellow, with a dark median area at lower border, median dark stripe usually wanting; tergum 3 orange, with a broad median black stripe, often extending a little along anterior margin, bordered with narrow to broad silvery stripes, and always without silvery marginal spots; tergum 4 with a narrow median black stripe edged with silvery stripes, brown admedian stripes and marginal silvery spots; tergum 5 with a median dark area edged with silvery or greyish-yellow stripes, marginal silvery spots.

MALE TERMINALIA. Figs. 3; 4. ANAL CERCI. Distal margin rounded, the inner ends not forming nipples. PHALLOSOME. Chitinous part long and narrow, and with deep waist, the distal end expanded; dorsal membrane joining distal with proximal part with numerous short bristles; posterior process long and well expanded at end. PARAMERE. Anterior part long and rather narrow, with 1 long and 4-5 shorter bristles; posterior part with a short, narrow, cone-like prominence. FIFTH STERNUM. Short and broad, the posterior processes short, narrow, bent inwards and well serrated.

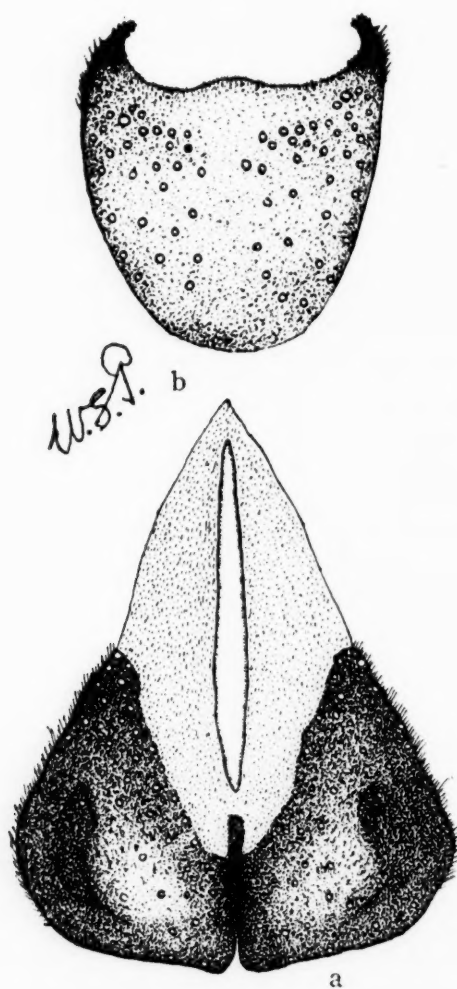


FIG. 4. *a.*—Anal cerci of *yerburyi*; *b.*—Fifth sternum.

FEMALE. HEAD. Vertex, wide, about three-quarters width of an eye; a single row of outer vertical hairs; cheeks grey. MESONOTUM. Similar to that of ♂. ABDOMEN. Orange with silvery markings; terga 1 and 2 similar to that of ♂; tergum 3 with a broad, black median stripe, merging into the spot on the lower border of terga 1 and 2 and extending a little along the upper margin of tergum 3, edged with broad silvery stripes, broad admedian brown stripes and well-marked silvery marginal spots; tergum 4 similar, except that the median black stripe is about half the width of that of the preceding tergum,

and the admedian brown stripes are narrower ; tergum 5 dark orange, sometimes darker in the middle, with silvery marginal spots.

EARLY STAGES. Oviparous ; commonly breeds about slaughter-houses in the green undigested matter from the stomach and intestines of cattle ; also breeds in horse and human faeces. THIRD LARVA. Anterior spiracles with 8-10 finger-like processes. POSTERIOR SPIRACULAR PLATES. Fig. 2, *b*. Rounded, wide, breathing slits long, narrow and well convoluted.

NOTES. This species was first bred at Saidapet, Madras, from the green material, referred to above, collected at the slaughter-house ; as far as I know it is found only in India. The ♂ terminalia are of peculiar interest, as the structure of the posterior part of the paramere suggests that it is a link between the *domestica* and *sorbens* groups. It is a common bazaar-fly.

SORBENS GROUP

Musca sorbens Wiedemann. For the synonymy, notes on the adults, and illustrations of the posterior spiracles of the third larva, see Pt. 3 ; for notes and illustrations of the ♂ terminalia, see Pt. 1. *Sorbens* is widely distributed throughout the region.

Musca vetustissima Walker. For the synonymy, notes on adults, illustrations of the ♂ terminalia and posterior spiracles of the third larva, see Pt. 3. *Vetustissima* is widely distributed in the region, and, like *sorbens*, is a common bazaar-fly.

Musca ventrosa Wiedemann. For the synonymy, notes on the adults, illustrations of the ♂ terminalia and posterior spiracles of the third larva, see Pt. 2. This species is widely distributed in the region.

Musca vitripennis Meigen. For the synonymy and notes on the adults, see Pt. 2 ; for illustrations of the ♂ terminalia, see Pt. 1. *Vitripennis* is found in Kashmir and possibly all along the northern limits of the region.

Musca fasciata Stein. For notes on the adults and illustrations of the ♂ terminalia, see Pt. 3. I have seen specimens of this small, dark, two-striped species from Calcutta, and Dr. Green sent me some from Malay. It is haematophagous in habit, and is found on animals and on cow-dung.

Musca tempestiva Fallen. For the synonymy, notes on the adults, and illustrations of the ♂ terminalia and the posterior spiracles of the third larva, see Pt. 2. *Tempestiva* is found in Kashmir.

Musca albina Wiedemann. For the synonymy and notes on the adults, see Pt. 2 ; for illustrations of the ♂ terminalia, see Pt. 1. *Albina* has been recorded from Ceylon and the north-west frontier of India.

Musca conducens Walker. For the synonymy, notes on the adults, and illustrations of the posterior spiracles of the third larva, see Pt. 3 ; for illustrations of the ♂ terminalia, see Pt. 1. The prestomal teeth, etc., are illustrated in another paper (1933a). It is widely distributed in the region, and is a common species on animals in the field.

Musca crassirostris Stein. For the synonymy, notes on the adults and illustrations of the posterior spiracles of the third larva, see Pt. 2; for illustrations of the ♂ terminalia, see Pt. 1. The proboscis, prestomal teeth, etc., are illustrated in another paper (1933a). This is the common blood-sucking species, and is a pest of cattle.

Musca lucens Villeneuve. Ann. Sci. Nat., Zool., V (ser. 10), 336, 1922.

MALE. HEAD. Vertex very narrow, eyes approximated; a large faceted area present on upper and inner side of eye. MESONOTUM. Two broad black stripes. ABDOMEN. Mainly yellow; terga 1 and 2 dark brown; tergum 3 yellow, with a median black stripe extending anteriorly and posteriorly to form narrow bands; tergum 4 yellow, with a median dark stripe and a dark posterior band; tergum 5 yellow. All sterna black. Male terminalia not examined.

FEMALE. HEAD. Vertex almost width of an eye; vertical stripe about one-third width of an eye.

MESONOTUM. Very similar to that of ♂. ABDOMEN. Orange; terga 1 and 2 orange, without markings; terga 3 and 5 very similar. Sterna black.

EARLY STAGES. Unknown.

NOTES. This small haematophagous species with two mesonotal stripes and black sterna was described by Villeneuve from Trincomali, Ceylon. The above short description is from specimens from Trincomali and Haragam, Ceylon, collected by the late Col. Yerbury, and now in the British Museum. I have not been able to get any specimens.

LUSORIA GROUP

Musca senior-whitei Patton. Ind. Jl. Med. Res., X, 73, 1922.

MALE. HEAD. Vertex very narrow, eyes approximated; cheeks silvery; palps light to dark brown and narrowly clubbed. Mentum of proboscis thickened; five long narrow prestomal teeth (see illustrations, 1933a).

MESONOTUM. Slate-blue, with four well-separated dark-brown stripes, the inner pair distinctly narrower than the outer, particularly in front of mesonotal suture, and ending abruptly some distance in front of scutellum; outer pair simulating triangular spots in front of suture, not reaching anterior margin, interrupted at suture and complete behind it, where they become broader; R_{4+5} with a row of bristles on ventral surface extending beyond radio-medial cross-vein. ABDOMEN. Mainly grey; terga 1 and 2 mainly black, consisting of a broad black median stripe, slate-blue band on each side extending narrowly to lateral margin; tergum 3 slate-blue, with broad black median stripe expanding at anterior end T-shaped, narrow dark-brown to black admedian stripes and silvery median patches; tergum 4 similar, but median stripe narrower; tergum 5 with narrow incomplete median stripe edged with blue patches, admedian black stripes and marginal silvery spots.

MALE TERMINALIA. Fig. 5. ANAL CERCI. Rather narrow, outer margin rounded, inner forming prominent finely haired nipples. PHALLOSOME. Chitinous

part short; membrane joining distal with proximal part with fine spines; posterior process long, bent and deeply forked. PARAMERE. Anterior part short, wide and bluntly pointed, and with either one long and two shorter bristles, or only one long and one short bristle; posterior part not very long, wide, and sometimes with end constricted, as in illustration. FIFTH STERNUM. Short and wide; posterior processes short, narrow, and bent inwards.

FEMALE. HEAD. Vertex wide, almost equal to width of an eye; vertical stripe black, about one-third width of vertex; outer vertical hairs long and in a single row, with smaller ones on outer side at lower end. Palps and proboscis

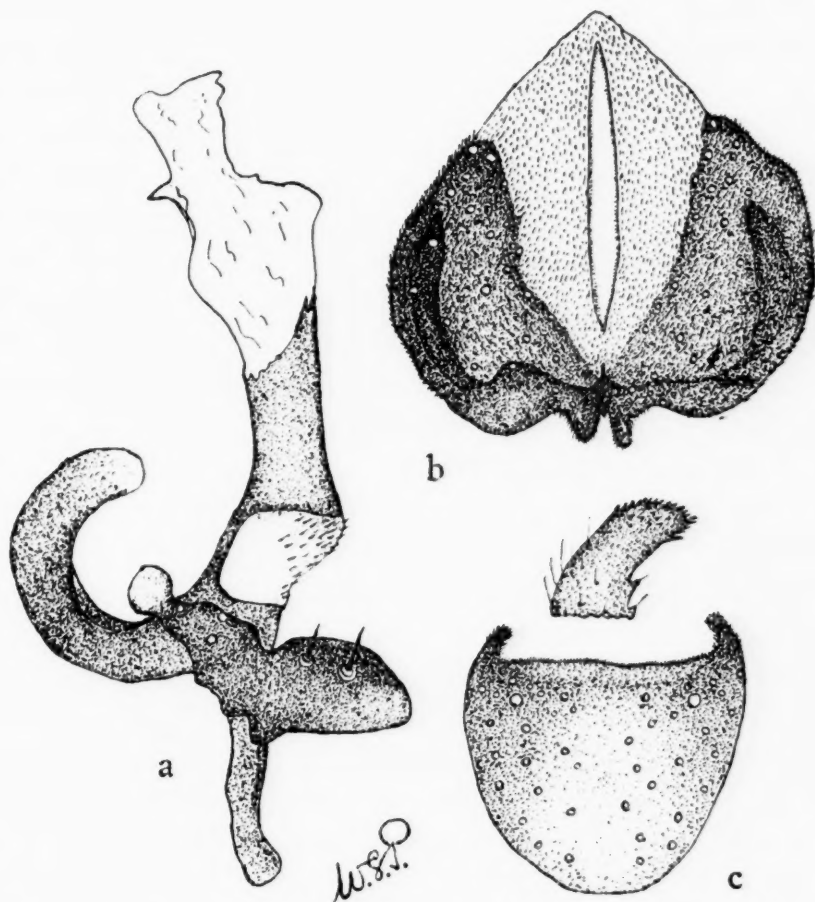


FIG. 5. *a*.—Phallosome and one paramere of *senior-whitei*; *b*.—Anal cerci; *c*.—Fifth sternum.

as in ♂. MESONOTUM. As in ♂. ABDOMEN. Markings very similar to those of ♂, except that the blue bands on terga 1 and 2 and also median stripes are wider; tergum 5 has no median stripe.

EARLY STAGES. Unknown.

NOTES. This species was first collected on cattle in 1913 on the shores of the canals of the Krishna River and on the islands of Kollar Lake, Bezvada District, Madras. Judging from the structure of the proboscis, illustrated and noted in another paper (1933*a*), it is most probably a blood-sucker. It is widely distributed in Malay, Sumatra, Java and the Philippine Islands. I have recently

had the opportunity of examining some specimens collected by Mr. Ho from the Island of Hainan. It probably occurs on the mainland in south-west China. The ♂ terminalia suggest that it is a link between the *sorbens* and the *lusoria* group, the posterior part of the paramere being relatively short.

Musca craggi Patton. Ind. Jl. Med. Res., X, 75, 1922.

SYNONYM: ? *pulla* Patton nec Bezzi.

MALE. HEAD. Vertex narrow, from one-sixth to one-eighth width of an eye; cheeks silvery. MESONOTUM. Silvery to bluish-grey, with four black stripes, the admedian pair narrow anterior to the suture, widening out behind; external pair broad, triangular-shaped before suture, interrupted and widening out posterior to suture; anterior portion of scutum with much silvery pollen, and central light-grey stripe well marked. ABDOMEN. Orange, with silvery and black markings; terga 1 and 2 black; tergum 3 orange, with a broad median dark-brown stripe which spreads out along the anterior border, lateral margins silvery; tergum 4 orange, with a narrower median black stripe, and a comparatively broad black median band at the posterior margin, lateral margins with much yellow pollen; tergum 5 dark orange, with a wider median black stripe, and two well-marked greyish-yellow spots at sides; apex of tergum grey.

MALE TERMINALIA. Illustrated in Pt. 1. ANAL CERCI. Narrow, with well-marked nipples on inner border, a deep emargination between nipple and outer margin, which is rounded. PHALLOSOME. Chitinous part rather short and with slight waist; posterior process long, markedly curved and widely forked at end. PARAMERE. Anterior part long and narrow, with one long and two shorter bristles; posterior part long, bent at end, with several long sensory hairs. FIFTH STERNUM. Long and wide, with a long dark area at each side of the distal end; posterior processes long, narrow and black.

FEMALE. HEAD. Vertex about equal to width of an eye; a single row of outer vertical hairs. MESONOTUM. Very similar to that of ♂. ABDOMEN. Dark orange; terga 1 and 2 dark-brown to black; tergum 3 dark orange, with silvery-yellow spots at lateral margins; tergum 4 similar, but lateral yellow spots more extensive; tergum 5 dark orange, with lighter margins.

EARLY STAGES. Unknown.

NOTES. This species was collected in 1921 in the Nilambur District, South India, on cattle and buffaloes. It is widely distributed in Ceylon and Malay.

Musca villeneuvei Patton. Ind. Jl. Med. Res., X, 74, 1922.

MALE. HEAD. Vertex very narrow, eyes approximated; cheeks silvery. MESONOTUM. Shining black, with two very broad black stripes with a light bluish-grey stripe between; a bluish-grey stripe at edge of scutum; R_{4+5} with a row of bristles on ventral side extending beyond radio-medial cross-vein. ABDOMEN. Dark orange, with dark and silvery markings; terga 1 and 2 dark orange, with a black median stripe; tergum 3 dark orange, with a broad dark median stripe, edged with yellow patches and marginal yellow spots, the posterior edge of segment dark brown; tergum 4 similar, but the median black stripe

narrower, and posterior margin with a wider dark band; tergum 4 with a very dark median stripe, edged with silvery stripes and marginal spots.

MALE TERMINALIA. Fig. 6. **ANAL CERCI.** Wide, with well-developed nipples on inner borders; outer border rounded. **PHALLOSOME.** Chitinous part rather short and broad, membranous portion long; posterior process long and deeply forked. **PARAMERE.** Anterior part long and broad, with one long stout bristle and two shorter ones; posterior process long, narrow and markedly

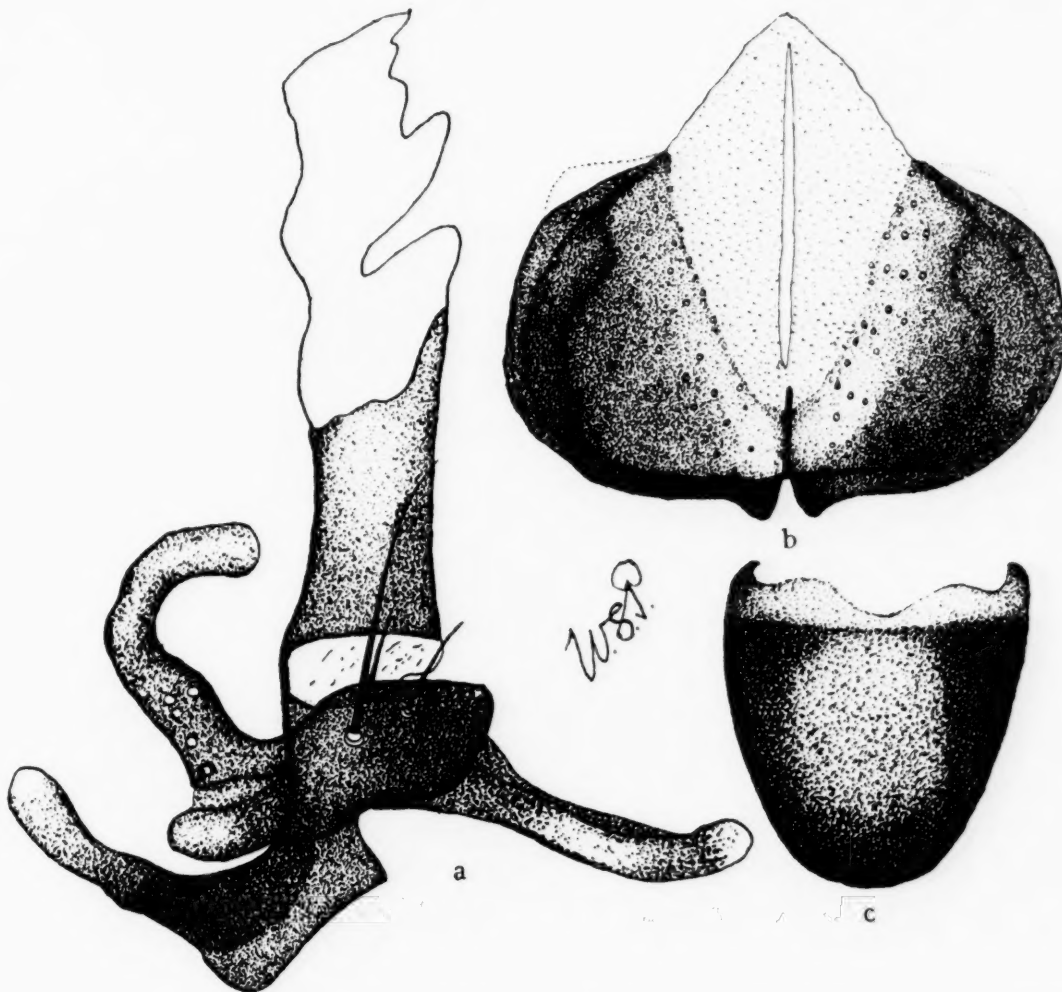


FIG. 6. a.—Phallosome and one paramere of *villeneuvei*; b.—Anal cerci; c.—Fifth sternum.

bent. **FIFTH STERNUM.** Fig. 6, c. Short and wide, the posterior processes very short.

FEMALE. **HEAD.** Vertex wide, a little more than half width of an eye; outer vertex hairs in a single row; vertical stripe wide, black with grey dusting; cheeks grey. **MESONOTUM.** As in ♂; the sides yellowish, R_{4+5} with bristles as in ♂. **ABDOMEN.** Dark orange—darker than that of ♂; terga 1 and 2 with a dark-brown band along posterior border; tergum 3 with a broad black band at its posterior border, and a broad black median stripe; terga 4 and 5 similar to that of ♂.

EARLY STAGES. Unknown. Puparium white.

NOTES. This species was bred from puparia collected from earth near elephant-dung at Nilambur, South India, in 1921. I have not seen it from any other part of India.

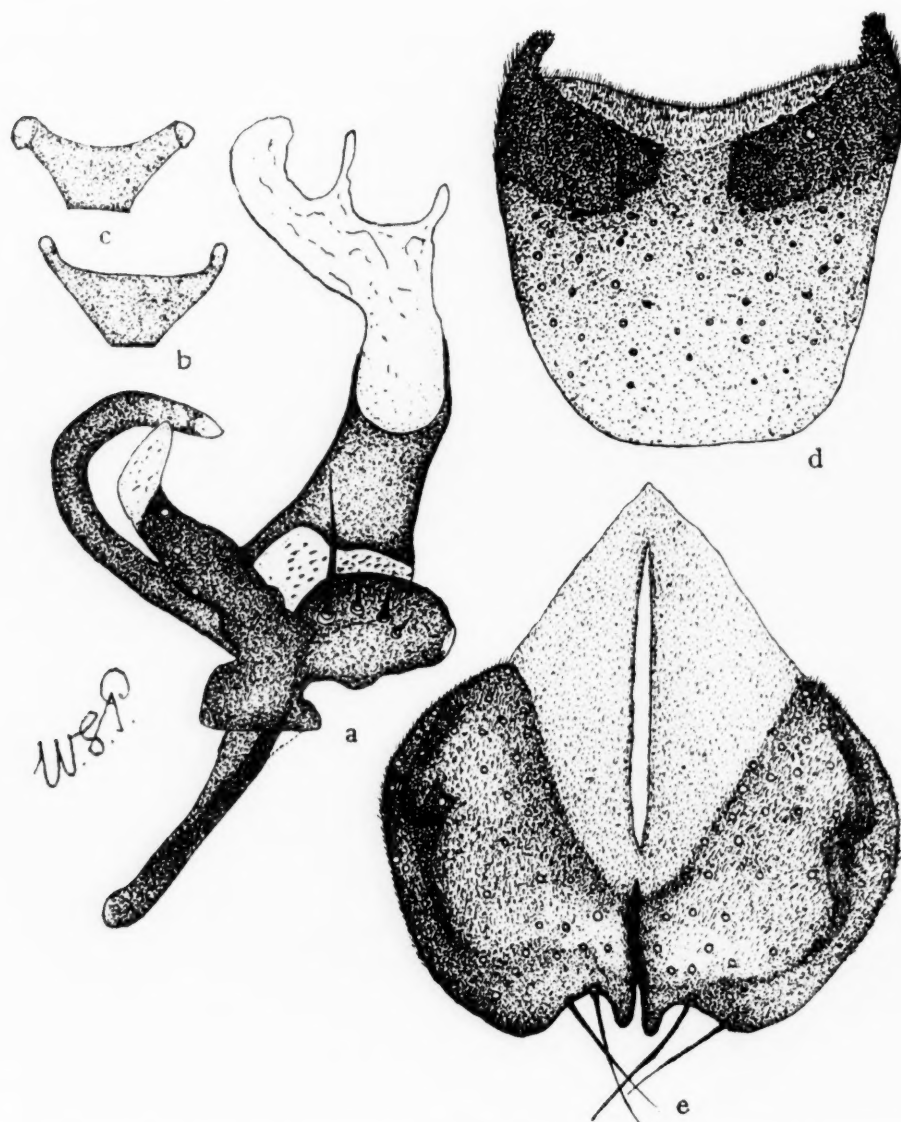


Fig. 7. *a*.—Phallosome and one paramere of *gibsoni*; *b*.—Ventral view of posterior process of phallosome; *c*.—Dorsal view of same; *d*.—Fifth sternum; *e*.—Anal cerci.

Musca gibsoni Patton and Cragg. Ind. Jl. Med. Res., I, 14, 1913.

SYNONYM: ? *latifrons* Awati.

MALE. HEAD. Vertex narrow, eyes approximated, and with fine scattered hairs. MESONOTUM. Four broad black stripes, R_{4+5} with a row of bristles extending beyond the radio-medial cross-vein. ABDOMEN. Dark orange, with black and silvery markings; terga 1 and 2 dark brown, the sides and posterior border light orange, a wide dark-brown median stripe; tergum 3 orange, with a broad black median stripe, adjacent silvery stripes and silvery marginal spots;

tergum 4 similar, but median stripe narrower, admedian dark stripes well marked, and forming an almost complete posterior dark band; tergum 5 with a narrow median dark stripe, broad adjacent silvery stripes, dark-brown admedian stripes and silvery margins.

MALE TERMINALIA. Fig. 7. ANAL CERCI. With well-marked, long, rather narrow nipples at inner border, outer border rounded, with a deep emargination between it and nipple. PHALLOSOME. Chitinous part short and wide, membranous portion long; many short spines on membrane joining proximal and distal part of phallosome; posterior process very long and bent upwards, with wide, shallow fork at end. PARAMERE. Anterior part short, with

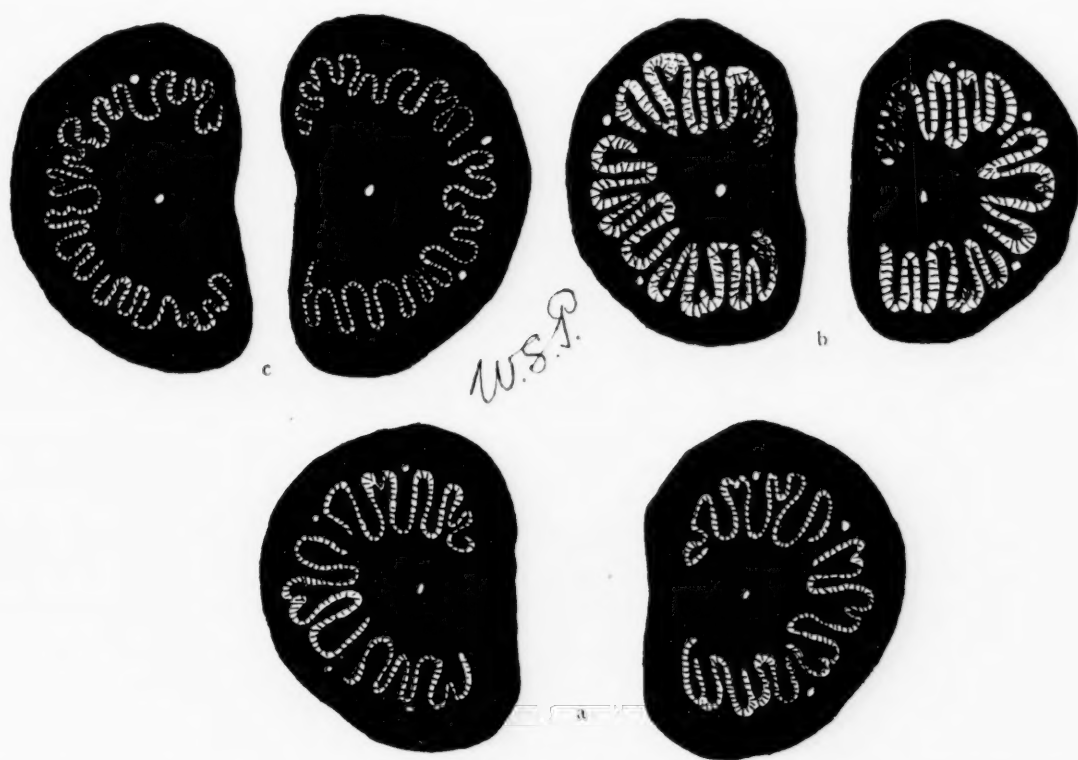


FIG. 8. *a*.—Posterior spiracles of third larva of *gibsoni*; *b*.—Same of third larva of *bezzii*; *c*.—Same of third larva of *pattoni*.

one long stout bristle and three smaller ones; posterior part long and wide and bent at the end. FIFTH STERNUM. Long and broad, with long lateral dark patch at each side of distal end; posterior processes wide, bent in and well serrated.

FEMALE. HEAD. Vertex wide, about three-quarters width of an eye; outer vertical hairs in two rows; eyes with scattered minute hairs, which can only be seen with a high power. MESONOTUM. As in ♂; R_{4+5} also with a row of bristles as in ♂. ABDOMEN. Dark orange, with black and silvery markings; terga 1 and 2 orange, anterior and posterior borders dark brown to black, and a broad black median stripe, sometimes incomplete; tergum 3 dark orange, with a narrow median dark-brown stripe spreading out widely along

anterior border, dark-brown admedian stripes and silvery marginal spots; tergum 4 similar, but the dark stripes much narrower, marginal spots yellowish; tergum 5 mainly silvery, with a broad dark median stripe, and a narrow admedian stripe not reaching anterior border.

EARLY STAGES. Oviparous, eggs laid singly in patches of cow-dung, the stalk of the egg, which is long and stout, directed uppermost. THIRD LARVA. Anterior spiracles with 6-7 finger-like processes. POSTERIOR SPIRACULAR PLATES. Fig. 8, *a*. Large, densely chitinated and widely separated, breathing slits narrow, long and much convoluted.

NOTES. This medium-sized haematophagous species is widely distributed in south India, at elevations of 4,000 feet and upwards. It also occurs in Assam and Sikkim. It is found only on animals and on and about patches of cow-dung in the field.

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(To be continued)

STUDIES IN RURAL HYGIENE IN THE TROPICS

BY

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IV.—THE PLACE OF MASS TREATMENT IN TROPICAL HYGIENE

(Received for publication 5 February, 1937)

The term 'treatment' usually implies that the cause of some disease or infection is definitely known to be present in the individual at the time when the drugs or other remedies are administered. Diagnosis being an essential preliminary, it does not alter the principle whether only one individual or a group of a thousand persons at a time is being treated.

The expression 'mass treatment,' however, brings quite a different conception into medical practice. By mass treatment most people understand the administration, to a group of persons, of remedies appropriate for the treatment of some particular disease or infection, but without the specific diagnosis having been made in each case. Some, or it may be many, of the individuals of the group have not been proved to be, and quite probably are not, in fact, sufferers from the disease or infection for which the remedies are being applied. We may illustrate the matter in an extreme way: if 100 people, in each one of whom ancylostomiasis has been correctly diagnosed, are given a suitable curative dose of carbon tetrachloride, that is treatment; if, on the other hand, 100 people are given a similar dose of carbon tetrachloride, when a diagnosis of ancylostomiasis has been reached in only 50 of them, that is mass treatment.

Mass treatment appears also to differ in principle from 'blunderbuss' treatment. In the blunderbuss method the diagnosis is properly made, and the object aimed at is actually known to be present. A varied assortment of missiles of debatable value is then discharged at it, in the hope that one or other of them will take effect. When mass treatment is adopted, the proper missile suitable for hitting a specific object is at hand; what remains doubtful is whether the object is there to be hit.

The employment of mass treatment has been recommended for two fundamentally different reasons. On the one hand, the method is brought into operation as a measure intended solely to relieve the symptoms of any possible patients. The remedies are administered with generous indiscriminate-ness to everybody, in order that those people in the group who actually happen to be suffering from this disease or infection may benefit as individuals; this is called the curative aim of mass treatment. On the other hand, the method is also adopted because the claim is put forward that the cure of the infected individuals during the course of mass treatment is recognized as an extremely important measure in curtailing the spread of disease.

In the case of malaria, the idea has been abandoned that quinine acts as a

true 'prophylactic' by preventing infection. Nowadays the utmost claimed for regular quinine-taking is that it keeps down an infection or a series of infections to such a low level that the clinical manifestations are at a minimum. This being so, it appears that, in the prophylactic use of quinine, we are really putting into effect mass treatment of an extreme kind. None of the people who take the drug are actually infected with malaria when they commence dosing themselves, some of them remain free from it, others acquire infection and may later be cured, while the rest will acquire and retain infection, though they may or may not develop clinical manifestations. The method of regular quinine administration, although it can apparently be classified as a form of mass treatment, carried out in intelligent anticipation, is not regarded by most people as dangerous or as necessarily undesirable.

The reason why the method of mass treatment may be considered permissible for one disease, and yet for another be held to be quite unjustifiable, lies not so much in the disease or in the method, as in the choice of remedy and the relative danger to human life of the particular drug employed. Quinine happens to be a drug of comparatively low toxicity, so that large doses may be taken without permanent ill-effect. But it is quite otherwise with some drugs which have nevertheless been used largely for mass treatment in other diseases. Carbon tetrachloride has produced many fatal results, not only in the genuine treatment of diagnosed cases of ancylostomiasis, but also in the course of mass treatment. The death of even a single person, who turns out after all not to have been infected, must weigh heavily in the scales against any benefits those other persons, who did happen to be infected, may have derived from the wholesale procedure.

In these days it may be said with justice that it is not scientific to administer any remedy for the eradication of ancylostomiasis infection, unless it has been proved by microscopic or other equally sound evidence that ancylostome infection actually exists in the person concerned. Exactly the same argument applies to the administration of remedies for any other diseases which can be accurately diagnosed. However, the most powerful argument against mass treatment is not the theoretical one, namely, that it is unscientific, but the severely practical one, that it will be dangerous to the life of the individual, if certain potent remedies are employed.

Should drugs and methods of administering them be discovered which are without any danger to the life of the uninfected individual, then, although the mass treatment of ancylostomiasis or any other disease must still be classed as unscientific, there will, in practice, be little objection to its adoption, whether as a curative or a preventive procedure.

Before the use of any drug can be recommended for mass treatment, it seems essential that, as a minimum requirement, the drug should have been proved to be safe when administered to diagnosed cases of the disease.

During my visit to Assam, Dr. Manson, at Cinnemara, provided some

figures which showed a good record of safety in a trial of tetrachlorethylene for ancylostomiasis ; this drug is also being tested extensively elsewhere. In this series, certain definite precautions were being taken, the coolies being examined and put into hospital for at least one day during the administration. Eight hundred cases had been treated, not only with no deaths, but without any troublesome symptoms. The coolies only said that they felt a little drunk and asked for a drop more. Further experience of such a drug and such methods of administering it with due precautions in long series of cases may eventually prove that it is so safe that it could be recommended widely for mass treatment.

No doubt, if drugs are found for which it can truthfully be claimed that they do not cause the death or invaliding of uninfected persons, this may make their employment quite justifiable in mass treatment for curative purposes. Whether any similar justification for their use with a view to the prevention of the spread of disease can be deduced therefrom, will depend on many circumstances, and especially on local conditions.

In Ceylon, Dr. Koomainswamy was carrying out treatment of school-children with oil of chenopodium and carbon tetrachloride ; the children took their doses very willingly, and even the parents were coming in to receive their share. Where such treatment is conducted on a voluntary basis so far as the patients are concerned, much work of a preparatory nature is frequently required. Taking school-children for example, the parents have to be told that there is infection among the children ; this alone involves a good deal of preliminary examination and diagnosis of faecal samples. They have to be informed also that the infection is the cause of the obvious ill-health present, and that treatment will not only be effective in curing the symptoms in their child, but will also be quite safe.

If, however, the administration is to be on a mass treatment basis, many parents must be told that it is by no means certain that their child is infected, and that no improvement in health can be hoped for if the child is really not infected. This information will in all probability give rise to quite grave doubts in the parent's mind as to the necessity for the treatment at all. Then comes the actual administration, involving some days' interruption of school-work on the part of the teachers and children, and also the expenditure of time and trouble, both by the doctors and the dispensers. Even so, there are apt to be serious omissions in voluntary treatment : in the case of school-children, for example, small children are often omitted, as are those who cannot be persuaded to attend ; parents and relatives are also largely excluded, so that the mass treatment of school-children does not by any means ensure that all the probably infected in any family or house receive their dose.

In some schools, the mass treatment has become an annual event, and this phenomenon at once suggests the following train of thought. If those who were infected at the previous annual administration are infected this year, then either they were not cured by the former dosage, or else they have re-acquired infection

since. Alternatively, if the children are not infected now, whether because they have never been infected at all, or because they were cured at the previous treatment, then they are receiving the treatment quite unnecessarily.

In so far as mass treatment is to be employed with the idea of preventing the spread of disease or of finally eradicating it, we ought, I think, to regard the method very critically. Several questions should be put to anyone proposing to recommend it for this purpose. What is the evidence, derived from the locality in which mass treatment is to be carried out, that the disease would be so fatal to those who suffer from it as to justify the unethical procedure of endangering the lives of uninfected persons? Is the treatment to be given to such a number as will include the vast majority of those who are infected in the village or district? If not, what will prevent widespread reinfection being derived from those who remain untreated? As regards the treated themselves, what precautions have been taken to ensure that one of the chief results of the treatment will not be an intensification rather than a diminution of soil contamination?

Even a great freedom from accidents in large series of persons mass-treated—and there have been large series recorded—should not divert our attention from the basic consideration that, as long as insanitary conditions in the villages are allowed to remain such as to favour soil and similar infection, the fundamental problem remains unsolved.

The ultimate preventive value of any mass-treatment method for ancylostomiasis and other excretally spread diseases depends chiefly on two considerations: the first is that all those capable of spreading infection shall be successfully treated, and the second is that the excreta of the treated shall be disposed of safely.

If the local rural methods of sanitation have already reached or can be brought up to this standard of safe disposal, then the method of mass treatment, in so far as its object is the prevention of spread of the infection by the agency of the excreta, has lost any significance, so that it and all risks attendant on it can be dispensed with.

Regarding the prevention of disease, it would appear much more satisfactory to consider each disease on its merits, taking into consideration especially the local conditions. Where the local rural difficulties opposed to practical methods of sanitation appear very formidable, and where the population is under control, a short cut in the shape of mass treatment for excretally spread diseases may appear very tempting. But to bring a free group up to the stage of the voluntary acceptance of even a moderately successful mass treatment, necessitates a large amount of patient education and much persuasion.

We ought in these days to be able to produce a more rational and permanent solution, by an equal effort in education, directed towards teaching the people in rural areas how to provide themselves with safe latrines, and how to use and look after them. Mass treatment with dangerous drugs should be regarded as the last resort, to be sought only when mass education in hygiene has been given a thorough trial and has demonstrably failed.

STUDIES IN CHEMOTHERAPY*

XIII.—THE CHANGES OBSERVED IN *T. BRUCEI* DURING FIVE YEARS' MAINTENANCE IN THE LABORATORY

BY

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AND

WARRINGTON YORKE

WITH AN APPENDIX BY

J. F. CORSON

(Received for publication 5 December, 1936)

Almost at the commencement of our experimental work on chemotherapy we were impressed with the fact that our laboratory strain of *T. rhodesiense*, which had been maintained in mice since it was isolated from man in 1923, was extremely sensitive to arsenicals; indeed, it was just as sensitive as were our laboratory strains of *T. equiperdum* and *T. gambiense*. This observation appeared to us to be especially noteworthy in view of the well-known fact that *rhodesiense* infections in man are resistant to arsenical therapy. There seemed to us to be two possible explanations for this curious phenomenon: either that the mere transference of *T. rhodesiense* from man to the mouse had resulted in an immediate increase in the susceptibility of the parasite to arsenicals, or that the increase in sensitiveness had developed gradually during the passage of the strain from mouse to mouse by means of the syringe.

With the object of investigating the matter, we decided to examine a number of freshly isolated strains from cases of *rhodesiense* infection of man in East Africa. In 1931, Dr. Corson, of Tanganyika, was kind enough to send us guinea-pigs inoculated from three recently diagnosed and untreated cases of sleeping sickness. From each of these guinea-pigs a number of mice was inoculated, and the sensitiveness of each strain to various arsenicals was determined.

As mentioned in previous papers (1934 and 1935), each of these three Tanganyika strains was, relatively to our old laboratory strain of *T. rhodesiense*, definitely resistant to arsenicals in that the infected mice could be cured only by the largest tolerated doses of the various drugs. If we regard the three strains as typical of untreated *rhodesiense* infections of man in East Africa, these observations afford an adequate explanation why aromatic arsenicals fail in the therapy of human *rhodesiense* infections. Moreover, they afford a strong indication that the sensitiveness of our old *rhodesiense* laboratory strain to arsenicals was not due to a sudden alteration consequent upon the transference of the

*This work was assisted by a grant from the Chemotherapy Committee of the Medical Research Council.

parasite from the human host to the mouse. It seemed, therefore, probable that the sensitiveness of this old strain must have been gradually acquired during its prolonged passage through mice in the laboratory. Some support for this hypothesis was obtained from a re-examination of the response to arsenicals of each of the three Tanganyika strains after they had been passaged through mice for a period varying from 8 to 10 months. In each case the strain was found to have become definitely more sensitive to arsenicals. It was also noticed that, whereas the duration of life of the mice inoculated from the original guinea-pigs infected from man varied from 3 to 6 weeks, after the strains had been passaged through mice for 8 to 10 months, the duration of life of the infected mice was only 1 to 2 weeks. It appeared, therefore, that the increase of sensitiveness to arsenicals was accompanied by an increase in virulence of the trypanosomes for mice.* It was this work which led us in 1933 to write: 'Recent observations have suggested to us that the manner in which a strain has been previously passaged in laboratory animals may exert a considerable influence on the results obtained in experiments, the object of which is to determine the response of that strain to therapeutic substances' (Yorke *et al.*, 1933b).

Preliminary observations of this nature caused us to make a careful analysis of the changes produced in a trypanosome by prolonged maintenance in the laboratory. For this purpose we selected *T. brucei*, which Dr. A. R. D. Adams had isolated in April, 1931, by exposing a dog in South Ankole, Uganda, to the bite of wild *G. morsitans*. This trypanosome was then maintained by passage through a series of guinea-pigs, the third of which was sent to England. As is shown in the accompanying plan, the parasite since it was received by us in August, 1931, has been maintained in a number of different ways.

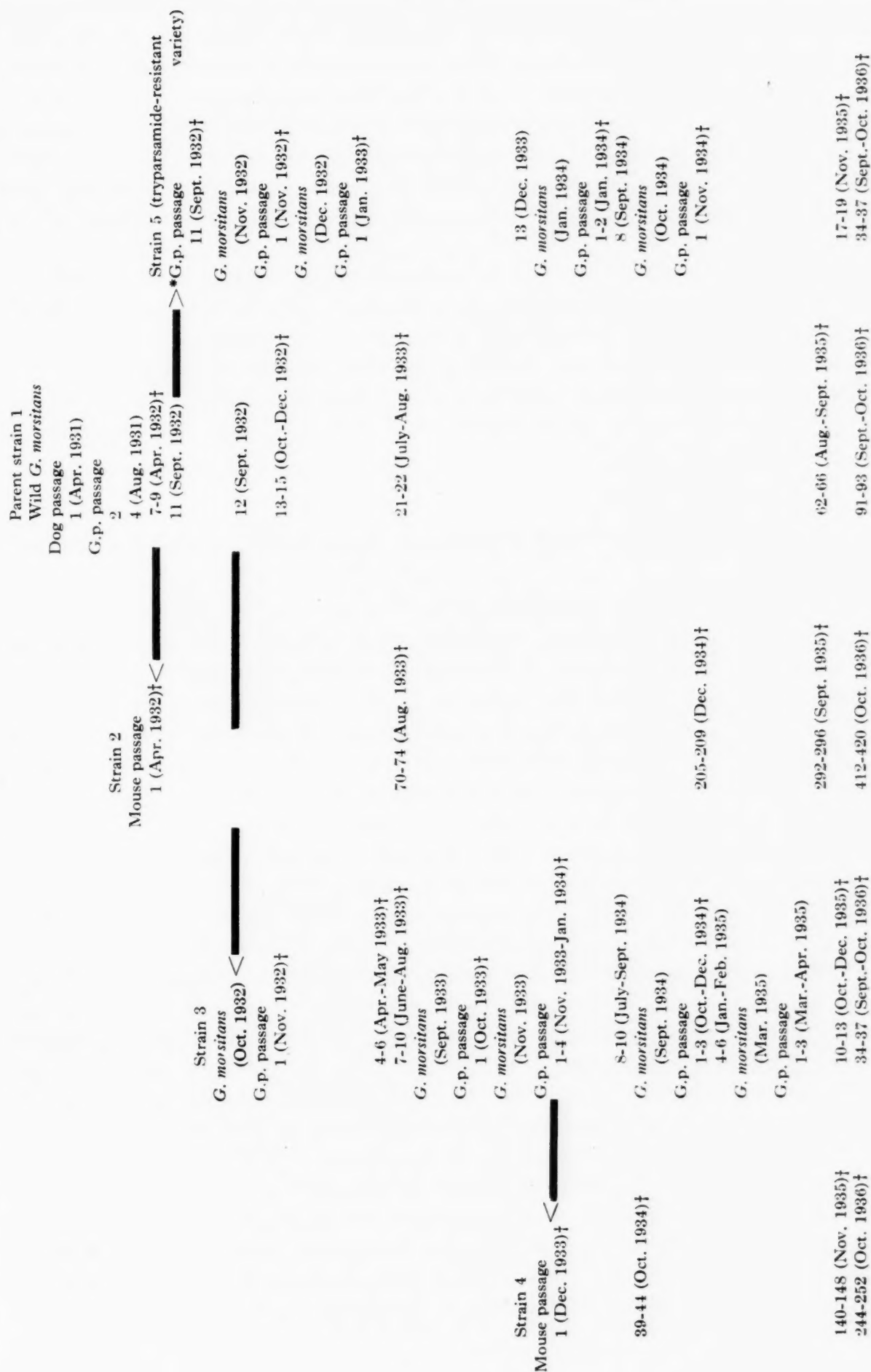
Strain 1. This was maintained by passage from guinea-pig to guinea-pig by means of blood inoculation. It constitutes the main parent strain from which the following originated.

Strain 2. This originated in April, 1932, by the inoculation of a mouse from the 7th guinea-pig of Strain 1; it was maintained by passage from mouse to mouse by means of blood inoculation.

Strain 3. This originated in September, 1932, from *G. morsitans* infected from a guinea-pig of the 12th passage of Strain 1; it was passaged through guinea-pigs by blood inoculation, interrupted as frequently as possible by cyclical transmission through *G. morsitans*.

Strain 4. This originated in December, 1933, by inoculating a mouse from a guinea-pig of Strain 3, after the trypanosome had been passed three times through *G. morsitans*: it was maintained like Strain 2 by direct blood inoculation through mice.

*A somewhat similar conclusion has recently been reached by Browning and Gulbransen (1935), who showed that a strain of *T. brucei* (the same strain as the one used by us in the work recorded in this paper) became more sensitive to therapeutic substances after prolonged passage through mice.



* The trypanosome was made arsenic-resistant by the repeated administration of subcurative doses of trypanamide. † Response to arsenicals tested.

Strain 5 (tryparsamide-resistant variety). This originated in September, 1932, when a guinea-pig of the 11th passage of Strain 1 was given numerous sub-curative doses of tryparsamide, as described in a previous paper (1933a), with the result that the infection became highly resistant to arsenicals. *G. morsitans* were infected with this arsenic-resistant strain, and transmitted it to guinea-pigs. The strain has, like Strain 3, since been maintained in guinea-pigs, partly by direct blood inoculation and partly by cyclical transmission through *G. morsitans*.

The objects we had in view in undertaking this lengthy piece of work were to ascertain whether any changes were produced in the trypanosome during a period of maintenance in the laboratory, and, if so, whether the changes were in any way determined or modified by the method of maintenance. The characters we have had particularly in mind were morphology, pathogenicity for guinea-pigs and mice, susceptibility to arsenicals and transmissibility by *G. morsitans*.

CHARACTERS OF THE FRESHLY ISOLATED TRYPANOSOME

When the parasite was received in this country in August, 1931, in the guinea-pig of the 3rd passage, 4 months after isolation from wild *G. morsitans*, it was typically polymorphic and exhibited numerous posterior-nuclear forms. The average duration of the infection in 5 guinea-pigs was 91 days; mice subinoculated from guinea-pigs of the 7th passage died on an average in 37 days. Trypanosomes were usually to be found in considerable numbers in the peripheral blood of both guinea-pigs and mice throughout the course of the disease.

The susceptibility to arsenicals was examined in mice inoculated from the guinea-pig of the 7th passage. It was found that the minimum effective dose (M.E.D.) of tryparsamide was about 8 mgm., that of reduced tryparsamide about 0.08 mgm., and that of halarsol about 0.03 mgm. per 20 gm. mouse. Cures, however, were only obtained with about the maximum tolerated dose (40 mgm.) of tryparsamide; maximum doses of reduced tryparsamide (1.5 mgm.) and of halarsol (0.32 mgm. per 20 gm. mouse) failed to cure. A very extensive series of therapeutic tests was performed in mice inoculated from guinea-pigs of the 13th to 15th passages. These are fully recorded in a previous paper (1933b), from which it can be seen that the M.E.D. of tryparsamide was about 8 mgm., of reduced tryparsamide about 0.08 mgm., of arsacetin about 3.0 mgm., and of halarsol about 0.03 mgm. per 20 gm. mouse. Only occasional cures were obtained with the largest tolerated doses of these drugs.

Although the capacity of the trypanosomes to develop in *Glossina morsitans* was not tested at the time of its arrival in Liverpool, there is no reason to doubt that it was really transmissible at that time, because *G. morsitans* were easily infected with it in September, 1932, i.e., 12 months later.

CHANGES EXHIBITED BY THE VARIOUS STRAINS DURING 5½ YEARS' MAINTENANCE IN THE LABORATORY

Strain 1. Passed through guinea-pigs by blood inoculation. The changes experienced by this strain are summarized in Table I. The parasite

TABLE I

T. brucei (parent strain 1) : passed through guinea-pigs by blood inoculation

Date	Age of strain from isolation months	Guinea-pig passages after isolation	Pathogenicity in guinea-pigs days	Pathogenicity in mice inoculated from guinea-pigs days	Susceptibility in subinoculated mice to halarsol mgm. per 20 gm. mouse		Remarks
					M.E.D.	M.C.D.	
Apr. 1931		Wild <i>G. morsitans</i>					
July-Dec. 1931	3-9	3-5	91 [5]				
Mar.-June 1932	11-14	7-9	80 [5]	37 [5]	0.03	>0.32	Strain 2 mouse passage started from guinea-pig 7
Aug. 1932	16	10					
Sept. 1932	17	11					Strain 5 trypanamide-resistant branch started from guinea-pig 11
Sept. 1932	17	12					Strain 3 <i>G. morsitans</i> transmitted branch started from guinea-pig 12
Oct.-Dec. 1932	18-20	13-15	65 [6]			>0.32	
July-Aug. 1933	27-28	21-22	34 [5]			>0.32	
Nov. 1933-Jan. 1934	31-33	25-29	32 [5]				
July-Sept. 1934	39-41	38-42	28 [7]				
Oct.-Dec. 1934	42-44	43-48	24 [8]				
Mar.-Apr. 1935	47-48	51-54	24 [6]				
Aug.-Sept. 1935	52-53	62-66	22 [8]	11 [5]	0.03	0.16	
Feb.-Mar. 1936	58-59	75-78	21 [5]				
Sept.-Oct. 1936	65-66	91-93	24 [10]	13.5 [10]	0.02	0.16	Trypanosomes still polymorphic with posterior - nuclear forms

The figures in brackets indicate the number of animals upon which the average is based.

maintained in this manner exhibited no morphological changes. In September, 1936, it was still typically polymorphic and showed posterior-nuclear forms. Its pathogenicity for guinea-pigs steadily increased during the first three years of its maintenance; guinea-pigs of the 3rd-5th passages, 3-9 months after isolation, lived on an average 91 days, whereas those of the 43rd-48th passages, 42-44 months after isolation, lived only 24 days. Since that time the pathogenicity for guinea-pigs has remained practically unchanged. The pathogenicity of the strain for subinoculated mice shows a similar increase; mice inoculated from guinea-pigs of the 7th passage lived on an average 37 days, those inoculated from the guinea-pig of the 62nd passage lived 11 days, and those inoculated from the guinea-pig of the 92nd passage lived 13.5 days.

The susceptibility of the strain to arsenicals increased but slightly during its maintenance. Mice infected from guinea-pigs of the 7th, 13th and 21st passages were not cured by the maximum well-tolerated dose of halarzol, viz., 0.32 mgm. per 20 gm. mouse. Mice inoculated from guinea-pigs of the 64th passage, in September, 1935, 52-53 months after isolation of the strain, were cured by a dose of 0.16 mgm. halarzol per 20 gm. mouse, but only 1 of the 5 mice treated with 0.08 mgm. failed to relapse. The last test was performed in October, 1936, 66 months after isolation of the strain, on mice inoculated from a guinea-pig of the 92nd passage; all the mice given 0.08 mgm. or less of the drug per 20 gm. mouse relapsed, but all those given 0.16 mgm. or more were cured permanently.

G. morsitans were readily infected from the guinea-pig of the 12th passage, 17 months after isolation of the strain. We were not able ourselves to examine the infectivity for the fly of the trypanosomes in guinea-pigs of the later passages, but Dr. Corson, to whom we sent the strain in May, 1936, reports that he was unable to infect *G. morsitans* from guinea-pigs of the 84th and 85th passages, and from goats inoculated from guinea-pigs of the 86th passage (*vide* appendix).

Strain 2. Passaged through mice by blood inoculation. As will be seen from Table II, the trypanosome maintained in this manner exhibited most pronounced changes in all the characters under consideration. It lost its stout forms and posterior-nuclear forms and became typically monomorphic, exhibiting only long slender forms with free flagella.

The pathogenicity in mice rapidly increased. When first introduced into mice in April, 1932, it killed these animals in an average of 37 days. Mice of the 41st-46th passages, in April, 1933, died in about 7 days, whilst those of the 187th-191st passages, in October, 1934, died on an average in 5.5 days. Since that date the pathogenicity for mice appears to have been stabilized. A point of peculiar interest is the fact that after prolonged passage through mice the trypanosome had practically lost its power of infecting guinea-pigs. Of 2 guinea-pigs inoculated in December, 1935, from a mouse of the 322nd passage, neither became infected; of 2 inoculated in January, 1936, from a mouse of the 326th passage, 1 failed to become infected and the other became

infected, but lived for 144 days; a guinea-pig inoculated in April, 1936, from a mouse of the 352nd passage failed to become infected; and of 2 guinea-pigs inoculated in May, 1936, from a mouse of the 366th passage, 1 failed to become infected and the other developed a chronic infection and lived for 130 days. Thus, of 7 guinea-pigs inoculated from mice of the later passages, 5 failed to become infected and the other 2 developed very chronic infections lasting 144 and 130 days respectively. On turning to Table I we find that, at the time when Strain 2 was first introduced into mice, inoculated guinea-pigs invariably became infected, and that the average length of life was 80 days. It would therefore appear that prolonged passage of the trypanosome through mice

TABLE II

T. brucei (Strain 2): passaged through mice by blood inoculation; started in April, 1932, from guinea-pig 7 of Strain 1

Date	Age of strain from isolation months	Length of time in mice months	Mouse passages	Pathogenicity in mice days	Susceptibility in mice to halarzol mgm. per 20 gm. mouse		Remarks
					M.E.D.	M.C.D.	
April-May 1932	12-13	0-1	1-5	37 [5]	0.03	>0.32	
Oct.-Nov. 1932	18-19	6-7	17-20	19 [7]			
April 1933	24	12	41-46	7 [10]			
Aug. 1933	28	16	70-74	7 [11]		>0.16	
April 1934	36	24	133-137	6 [10]			
Oct. 1934	42	30	187-191	5.5 [10]			
Dec. 1934	44	32	205-209	5.3 [10]		>0.16	
April 1935	48	36	245-249	5.7 [10]			
Sept. 1935	53	41	292-296	5.4 [10]	0.02	0.04	
Mar. 1936	59	47	347-351	5.2 [10]			
Oct. 1936	66	54	412-420	5.8 [18]	0.02	0.08	
							Trypanosomes monomorphic, no posterior-nuclear forms

had definitely decreased its pathogenicity for guinea-pigs. It is interesting to note that microscopic examination of these two chronically infected guinea-pigs disclosed the fact that the parasite had regained its pristine morphological characters and had become once more typically polymorphic with posterior-nuclear forms. This polymorphism with posterior-nuclear forms was also maintained in mice inoculated from these two guinea-pigs, and the infection in these mice was distinctly less acute than it was in the mice immediately preceding the passage of the strain through the guinea-pig, viz., 12 days, as compared with 5.5 days. In contrast to this remarkable loss of pathogenicity for guinea-pigs, the strain was found to be acutely virulent for rabbits, as 2

rabbits inoculated from the mouse of the 366th passage developed an acute infection and died in 32 days.

The susceptibility of the strain to halarsol increased very markedly. When first introduced into mice, the infection was not cured by the maximum well-tolerated dose (0.32 mgm. per 20 gm. mouse) of halarsol. The sensitiveness of the strain to the drug had scarcely increased by December, 1934, but tests made in September, 1935, showed that the strain had become extremely sensitive to halsarol: of 6 mice treated with 0.04 mgm. per 20 gm. mouse, only 1 relapsed, and the same applies to 6 mice treated with 0.08 mgm. per 20 gm. mouse; all the mice treated with larger doses of the drug were cured. The last test was performed in October, 1936, 66 months after isolation of the strain in mice inoculated from a mouse of the 416th passage; all the mice given 0.04 mgm. or less of the drug per 20 gm. mouse relapsed, as did 1 of the 5 given 0.08 mgm.; all those given 0.16 mgm. or more were permanently cured.

TABLE IIA

Showing the results of attempts to transmit Strain 2 by *G. morsitans*, before and after passage through mice

Date of attempt to transmit	Temperature at which flies were kept	No. of flies used	Passage from previous fly transmission	Result of subsequent feeds on normal animal	No. of flies alive 11 days after 1st infecting feed	No. of flies found to be infected on dissection		
						Gut only	Gut and proventriculus	Gut, proventriculus and salivary glands
Sept. 1932	{ Day 60°-80° F. Night 82°-86° F.	89	G.-pig. 12 of strain 1*	positive	59	1	7	2
May 1936	{ Day 70°-86° F. Night 82°-86° F.	164	326†	negative	131	0	0	0

* This line shows the transmissibility of the strain about the time when it was first introduced into mice.

† Guinea-pig inoculated from mouse of 326th passage of Strain 2.

When first introduced into mice in April, 1932, the trypanosome was readily transmissible by *G. morsitans*. As shown in Table IIA, we were unable to infect *G. morsitans* by feeding them in May, 1936, on a guinea-pig inoculated in January, 1936, from a mouse of the 326th passage. Unfortunately, only 131 of the flies used in this test survived more than 10 days after the first infected meal, but as none of these on dissection were found to harbour any trypanosomes we concluded that the strain had actually lost its capacity for being transmitted by *G. morsitans*. This conclusion is supported by the observations of Dr. Corson, to whom the strain was sent in May, 1936 (*vide* appendix).

The changes experienced by the trypanosomes maintained by blood inoculation through mice are hence very pronounced; the morphology became

modified; the pathogenicity for mice greatly increased, and for guinea-pigs greatly decreased; the sensitiveness of the trypanosome to arsenicals was greatly enhanced; and the parasite became non-transmissible by *G. morsitans*. It is a very questionable matter whether some of these changes were as profound as they appeared to be, because a single passage through the guinea-pig sufficed to restore the original morphological characters of the strain and to reduce considerably its pathogenicity for mice.

Strain 3. Passed through guinea-pigs partly by blood inoculation and partly by *G. morsitans*. The changes exhibited by the trypanosome maintained in this manner (Table III) resemble those of Strain 1 in being relatively slight.

TABLE III

T. brucei (Strain 3): passed through guinea-pigs partly by blood inoculation and partly by *Glossina morsitans*

Date	Age of strain from isolation months	Guinea-pig passages after each fly transmission	Pathogenicity in guinea-pigs days	Pathogenicity in mice inoculated from guinea-pigs days	Susceptibility in subinoculated mice to halarzol mgm. per 20 gm. mouse		Remarks
					M.E.D.	M.C.D.	
Sept. 1932	17	12					<i>vide</i> Table 1
Oct. 1932		<i>G. morsitans</i>					
Nov. 1932	19	1	71 [7]	23 [5]	0.03	>0.32	
Apr.-May 1933	24-25	4-6	44 [4]			>0.32	
June-Aug. 1933	26-28	7-10	35 [5]			>0.32	
Sept. 1933		<i>G. morsitans</i>					Strain 4 mouse passage started from guinea-pig 1
Oct. 1933	30	1		20 [5]		>0.32	
Nov. 1933		<i>G. morsitans</i>					
Nov. 1933-Jan. 1934	31-33	1-4	54 [4]	21 [10]		>0.32	
July-Sept. 1934	39-41	8-10	52 [5]				
Sept. 1934		<i>G. morsitans</i>					
Oct.-Dec. 1934	42-44	1-3	60 [4]	16 [5]		>0.32	
Jan.-Feb. 1935	45-46	4-6	39 [5]				Trypanosomes still polymorphic with posterior - nuclear forms
Mar. 1935		<i>G. morsitans</i>					
Mar.-Apr. 1935	47-48	1-3	59 [5]				
Oct.-Dec. 1935	54-56	10-13	24 [8]	10 [5]	0.03	0.32	
Mar. 1936	59	20-22	20 [6]				
Sept.-Oct. 1936	65-66	34-37	19 [8]	10.5 [10]	0.02	0.08	

The parasite has undergone no morphological changes and is still typically polymorphic and shows numerous posterior-nuclear forms.

Whilst its pathogenicity for guinea-pigs and mice has increased to the same extent as Strain 1 (it now kills guinea-pigs on an average in 19 days and mice in 10.5 days), reference to Table III shows that the increase in pathogenicity

TABLE IIIA
Showing results of successive attempts to transmit Strain 3 by *Glossina morsitans*

Date of attempt to transmit	Temperature at which flies were kept	No. of flies used	Infected g.-pig on which flies fed. Passage from previous fly transmission	Result of subsequent feeds on normal animal	No. of flies alive 11 days after 1st infecting feed	No. of flies found to be infected on dissection		
						Gut only	Gut and proventriculus	Gut, proventriculus and salivary glands
Oct. 1932	{ Day 60°-80° F. Night 82°-86° F.	89	12	positive	59	1	7	2
Sept. 1933	{ Day 96° F. Night 82°-84° F.	213	10	"	58	1	10	1
Nov. 1933	{ Day 60°-75° F. Night 82°-84° F.	90	1	"	41	2	8	1
Sept. 1934	{ Day 60°-80° F. Night 82°-86° F.	179	10	"	79	0	4	4
Mar. 1935	{ Day 60°-80° F. Night 82°-84° F.	145	6	"	89	0	8	1
Jan. 1936	{ Day 60°-80° F. Night 80°-82° F.	237	13	negative	150	0	19	0
May 1936	{ Day 70°-86° F. Night 82°-86° F.	277	22	"	171	3	15	0
June 1936	{ Day 80°-98° F. Night 86° F.	287	26	"	58	0	0	0
July 1936	{ Day 82°-88° F. Night 82°-88° F.	615	29	"	435	3	0	0
Aug. 1936	{ Day 60°-90° F. Night 77° F.	163	31	"	130	0	0	0
Sept. 1936	{ Day 82°-86° F. Night 82°-86° F.	255	33*	"	64	0	0	0

* This was a sheep inoculated from a guinea-pig of the 33rd passage.

was neither so regular nor so rapid as in the case of Strain 1. The explanation of this fact appears to be that each passage of the strain through *G. morsitans* definitely reduced its pathogenicity for guinea-pigs.

The susceptibility of the strain to halarzol had scarcely changed up to December, 1935. At the commencement, subinoculated mice were not cured

by the maximum well-tolerated dose of halarsol, viz., 0.32 mgm. per 20 gm. mouse, whereas in December, 1935, subinoculated mice were cured by this dose, but relapsed after 0.16 mgm. per 20 gm. mouse. The last tests, performed in October, 1936, 66 months after isolation of the strain, on mice inoculated from a guinea-pig of the 36th passage after the 5th fly transmission, show that during the last year the trypanosome has become very sensitive to halarsol; 3 of the 5 mice given 0.04 mgm. of the drug relapsed, but all those given 0.08 mgm. or larger doses were permanently cured.

The results of successive attempts to pass this strain through *G. morsitans* are summarized in Table IIIA. It will be seen that between September, 1932, and March, 1935, the strain remained transmissible by *Glossina*. Between January and September, 1936, 6 transmission experiments were carried out. Although very large numbers of flies were used in these experiments, they all failed. The Table shows the results of dissecting all those which lived longer than the 10th day after the first infected feed. Although no salivary gland infections were discovered in the experiments conducted in January-May, 1936, it is interesting to note that a large number of gut and proventriculus infections were found; whilst in the experiments conducted in June-July, 1936, there were no proventriculus infections and only a very few gut infections. In subsequent experiments, even gut infections were not found. This work will be discussed in detail in a subsequent paper of this series, and we shall content ourselves here by concluding that they afford almost overwhelming evidence that the strain lost its capacity of being transmitted by *Glossina morsitans* some time after the 5th fly transmission between April, 1935, and January, 1936. The reason for this loss of transmissibility is unknown, but possibly it may be associated with the fact that owing to lack of *Glossina* we were unable to attempt to pass it through fly for a period of 8-9 months, during which the strain was transmitted by blood inoculation through a series of 13 guinea-pigs. This strain was also sent in May, 1936, to Dr. Corson, in Tanganyika, and, as shown in the appendix at the end of this paper, his observations confirm our conclusion that it had become non-transmissible by *Glossina morsitans*.

Strain 4. Originated in December, 1933, from a guinea-pig of Strain 3 after it had been passed 3 times through *Glossina morsitans*. Like Strain 2, it was then passaged by blood inoculation through mice. The changes exhibited by this strain (Table IV) are the same as those which occurred in Strain 2. It became monomorphic; its pathogenicity for mice increased greatly, and so did its susceptibility to arsenicals.

Strain 5 (tryparsamide-resistant variety). Originated in September, 1932, from a guinea-pig of the 11th passage of Strain 1. The infection in this guinea-pig was made highly resistant to tryparsamide by repeated administration of subcurative doses of the drug. The strain has since been transmitted through guinea-pigs by blood inoculation, interrupted from time to time by cyclical transmission through *G. morsitans*.

The original state of this strain is fully described in a previous paper (Yorke *et al.*, 1933b). It was typically polymorphic, with posterior-nuclear forms; of chronic pathogenicity for guinea-pigs and mice; completely resistant to aromatic arsenicals, in that maximum well-tolerated doses failed to clear the blood of parasites; and readily transmissible by Glossina.

The changes undergone by this strain are summarized in Table V. Its morphology has remained unchanged and it is still typically polymorphic, with posterior-nuclear forms.

The pathogenicity for guinea-pigs has increased from 71 days to 24 days, the increase following the same irregular course as that exhibited by Strain 3,

TABLE IV

T. brucei (Strain 4): passaged through mice by blood inoculation; started in December, 1933, from 1st guinea-pig after 3rd fly transmission of Strain 3

Date	Age of strain from isolation	Length of time in mice	Mouse passages	Pathogenicity in mice	Susceptibility in mice to halarzol mgm. per 20 gm. mouse		Remarks	
	months	months		days	M.E.D.	M.C.D.		
Dec. 1933-Jan. 1934	32-33	0-1	1-5	21 [10]	0.03	>0.32	This dose-0.16 mgm. -was the biggest given	
June 1934	38	6	22-26	22 [11]				
Oct. 1934	42	10	39-44	16 [10]		>0.16		
Dec. 1934	44	12	50-54	9.5 [10]	0.02	0.04-0.08		Trypanosomes monomorphic, no posterior-nuclear forms
June 1935	50	18	98-106	6 [19]				
Nov. 1935	55	23	140-148	6 [18]				
Mar. 1936	59	27	180-184	5.4 [10]	0.02	0.08		
Oct. 1936	66	34	244-252	5.0 [18]				

each transmission by *G. morsitans* tending to lessen the pathogenicity for guinea-pigs, so that it was only after the strain ceased to be transmitted by *G. morsitans* that the increase in virulence for guinea-pigs became pronounced. The virulence for subinoculated mice has likewise slowly increased from 44 to 15 days.

The acquired resistance to aromatic arsenicals has, however, remained unchanged, the strain being as completely resistant to halarsol and tryparsamide in October, 1936, as it was when it was first made resistant in September, 1932, maximum tolerated doses of the drug failing to exert the slightest influence on the infection.

TABLE V

T. brucei (Strain 5): tryparsamide-resistant branch passaged through guinea-pigs partly by blood inoculation and partly by *Glossina morsitans*; started in September, 1932, from guinea-pig 11 of Strain 1

Date	Age of strain from isolation months	Guinea-pig passages after each fly transmission	Pathogenicity in guinea-pigs days	Pathogenicity in mice inoculated from guinea-pigs days	Susceptibility in subinoculated mice to halarsol mgm. per 20 gm. mouse		Remarks
					M.E.D.	M.C.D.	
Sept. 1932	17	11			>0.6		The strain was made tryparsamide-resistant in this guinea-pig, <i>vide</i> Table 1
Nov. 1932	19	<i>G. morsitans</i>					
Nov. 1932		1	71 [3]	44 [5]*	>0.6		*Inoculated with metacyclic trypanosomes
Dec. 1932		<i>G. morsitans</i>					
Jan. 1933		1	73 [4]		>0.6		
May-June 1933		3-6	46 [4]				
Dec. 1933	32	13	37 [4]				
Jan. 1934	33	<i>G. morsitans</i>					
Jan. 1934		1-2	45 [4]	37 [3]	>0.6		M.E.D. tryparsamide >45.0 mgm.
Sept. 1934		8	40 [3]				
Oct. 1934	43	<i>G. morsitans</i>					
Nov. 1934		1	49 [1]	6 mice lived longer than 30 days	>0.6		
Jan. 1935	45	3	69 [3]				
Nov. 1935	55	17-19	33 [5]	21 [4]	>0.6		
Jan.-Feb. 1936	57-58	20-23	29 [7]				
Sept.-Oct. 1936	65-66	34-37	24 [9]	15 [5]	>0.6		Trypanosomes still polymorphic with posterior-nuclear forms

The results of successive attempts to transmit the strain by *G. morsitans* are shown in Table VA, from which it will be seen that the strain remained transmissible up to October, 1934. Experiments conducted in January-May, 1936, gave negative results, and dissection of the flies used in these experiments failed to reveal any evidence of infection. Corroborative evidence that the strain has become non-transmissible was obtained by Corson, in Tanganyika, to whom it was sent in May, 1936 (*vide* appendix).

TABLE VA
Showing the results of successive attempts to transmit Strain 5 by *Glossina morsitans*

Date of attempt to transmit	Temperature at which flies were kept	No. of flies used	Infected g.-pig on which flies fed. Passage from previous fly transmission	Result of subsequent feeds on normal animal	No. of flies alive 11 days after 1st infecting feed	No. of flies found to be infected on dissection		
						Gut only	Gut and proventriculus	Gut, proventriculus and salivary glands
Nov. 1932	{ Day 90° F. Night 82°-86° F.	160	1	positive	49	0	0	1
Dec. 1932	{ Day 90° F. Night 82°-86° F.	168	1	"	109	1	3	3
Jan. 1934	{ Day 96° F. Night 82°-84° F.	186	13	"	88	1	8	1
Oct. 1934	{ Day 60°-80° F. Night 82°-86° F.	257	8	"	153	2	10	2
Jan. 1936	{ Day 60°-80° F. Night 80°-82° F.	171	19	negative	113	0	0	0
May 1936	{ Day 70°-86° F. Night 82°-86° F.	285	26	"	182	0	0	0

The changes exhibited by this strain are thus, except in one important respect, similar to those exhibited by the normal Strain 3 maintained in the same manner. Its morphology has remained unchanged ; its pathogenicity for guinea-pigs and subinoculated mice increased slowly ; and it eventually lost its transmissibility by *G. morsitans*. It has, however, retained unimpaired its acquired character of arsenic-resistance.

DISCUSSION

These observations show that, whilst the various strains derived from the parent trypanosome all became modified during their 5½ years' sojourn in the laboratory, they did so to very different extents. The method of maintenance which produced the most rapid and the most pronounced modifications was that

of passage through mice by means of blood inoculation (Strains 2 and 4). This method of maintenance produced marked changes in all the characters studied by us. The trypanosome changed from a polymorphic to a monomorphic parasite; it became acutely pathogenic for mice, and almost entirely non-pathogenic for subinoculated guinea-pigs; it developed great sensitiveness to arsenicals, in that small doses of halarsol cured infected mice; and it lost its capacity of infecting *Glossina*.

The strains maintained in guinea-pigs changed much more slowly, and at the end of 5½ years their modifications were less pronounced. That transmitted partly by blood inoculation and partly by *G. morsitans* (Strain 3) changed even more slowly than that transmitted by blood inoculation (Strain 1); in fact, so long as the former remained transmissible by *Glossina*, which it did for a period of 4 years after isolation from wild *G. morsitans*, it exhibited no recognizable change, except that possibly its pathogenicity for guinea-pigs and subinoculated mice had slightly increased; since this period, it has, however, become modified in the same way as the latter strain. After 5½ years both strains were morphologically unchanged, the trypanosomes still being polymorphic and exhibiting numerous posterior-nuclear forms; their pathogenicity for guinea-pigs and subinoculated mice had, however, increased very definitely, as had their sensitiveness to arsenicals; and they had become non-transmissible by *Glossina*.

The changes exhibited by the arsenic-resistant variety (Strain 5), which was passaged through guinea-pigs, partly by blood inoculation and partly by *Glossina morsitans*, were in all respects but one strictly comparable with those exhibited by the normal variety passaged in a similar manner (Strain 3). So long as the strain remained transmissible by *G. morsitans*, which it did until October, 1934, its characters were practically unchanged. After it had become non-transmissible its virulence for guinea-pigs and subinoculated mice increased. In October, 1936, it resembled the normal varieties passaged through guinea-pigs (Strain 1 and 3) in that it was still polymorphic and exhibited numerous posterior-nuclear forms, its virulence for guinea-pigs and subinoculated mice had definitely increased, and it had become non-transmissible for *Glossina*. It differed from the normal strains, however, in one important respect, namely, that its resistance to arsenicals remained unimpaired, toxic doses of halarsol and tryparsamide failing to clear the blood of parasites even temporarily. The remarkable stability of this acquired character of arsenic-resistance will be discussed more fully in the next paper of this series.

SUMMARY

1. In view of the well-established fact that arsenicals are of little use in the treatment of human beings suffering from *rhodesiense* sleeping sickness, the extreme sensitiveness to these drugs of our old laboratory strain of *T. rhodesiense* in mice was very surprising.

2. Three strains of *T. rhodesiense* were obtained from recently diagnosed and untreated Tanganyika cases of sleeping sickness. These strains in mice of the first passage proved refractory to arsenical treatment, but in mice of later passages were definitely more susceptible.

3. These observations caused us to investigate experimentally the changes experienced by a trypanosome during its maintenance in the laboratory. The trypanosome used was *T. brucei* recently isolated from wild fly in Uganda. This parasite was maintained in four different ways during a period of 5½ years, and the changes were carefully observed. The characters considered were morphology, pathogenicity, susceptibility to arsenicals and transmissibility by *G. morsitans*.

4. It was found that all the four strains underwent modifications, but that those of the strains passaged through mice were much more rapid and pronounced than those of the strains passaged through guinea-pigs.

5. The mouse-passage strains exhibited changes of morphology; greatly increased pathogenicity for mice and greatly decreased pathogenicity for guinea-pigs; greatly increased sensitiveness to arsenicals; and loss of transmissibility by *G. morsitans*.

The guinea-pig-passage strains exhibited no change of morphology; they showed a gradual increase in pathogenicity for guinea-pigs and mice, and eventually a marked increase in sensitiveness to arsenicals, but this last change appeared more slowly than in the mouse-passage strains. These strains likewise finally lost their capacity of being transmitted by *Glossina*, but it must be noted that this character persisted for 4 years in Strain 3, which was passed from time to time through *Glossina*. So long as this strain remained transmissible by *Glossina*, its original characters appeared to be preserved practically unchanged.

6. A fifth strain which was made arsenic-resistant and passaged through guinea-pigs, partly by blood inoculation and partly by *G. morsitans*, behaved in exactly the same way as the corresponding normal variety, except that its acquired character of arsenic-resistance was maintained unimpaired.

We were unable to obtain any support for the hypothesis that the rendering of a trypanosome arsenic-resistant interferes with its capacity to develop in *Glossina*.

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APPENDIX

With a view to examining our conclusion that the various strains had actually lost the power of infecting and being transmitted by *Glossina*, they were sent to Dr. Corson, in Tanganyika, in May, 1936, i.e., 5 years after isolation of the parasite from wild *G. morsitans*. Dr. Corson confirms our conclusion and sends the following record of his observations.

TABLE I
Dr. Corson's observations on *T. brucei* (Strain 1)

Date	Age of strain from isolation months	Guinea-pig passages after isolation	Pathogenicity in guinea-pigs days	Pathogenicity in mice days	Remarks
May 1936	61	82	21 [2]	11 [10]	These guinea-pigs were sent from Liverpool to Dr. Corson
" "	61	83	22 [2]		Goats 11 and 12 inoc. from guinea-pig of 86th passage
June-July 1936	62-63	84-87	17 [7]		
Aug. 1936	64	88-90	22 [5]		

TABLE IA
Results of Dr. Corson's attempts to transmit Strain 1 by *Glossina morsitans*

Date of attempt to transmit	Temperature at which flies were kept	No. of flies used	Infected g.-pig on which flies fed Passage	Result of subsequent feeds on normal animal	No. of flies alive about 40 days after 1st infecting feed	No. of flies found to be infected on dissection	
						Gut only	Gut and salivary glands
June-July 1936	{ Laboratory conditions at Tinde, Tanganyika }	100-120	84-85	negative	25	0	0
Aug. 1936		250-300	Goats 11 and 12*	"	30	0	0
Sept. 1936		150-180	Goat 11*	"	79	0	0

* Inoculated from guinea-pig of 86th passage of Strain 1.

TABLE II

Dr. Corson's observations on *T. brucei* (Strain 2)

Date	Age of strain from isolation months	Guinea-pig passages after transfer from mice	Pathogenicity in guinea-pigs days	Pathogenicity in mice days	Remarks
Jan. 1936	57	1	144 [1]		This guinea-pig was inoculated from mouse of 326th passage of Strain 2 These animals were sent from Liverpool to Dr. Corson
May 1936	61	2	63 [2]		
June 1936	62	3-4	74 [2]	10 [9]	
Aug. 1936	64	5			

TABLE IIA

Result of Dr. Corson's attempt to transmit Strain 2 by *Glossina morsitans*

Date of attempt to transmit	Temperature at which flies were kept	No. of flies used	Infected g.-pig on which flies fed Passage	Result of subsequent feeds on normal animal	No. of flies alive about 40 days after 1st infecting feed	No. of flies found to be infected on dissection	
						Gut only	Gut and salivary glands
Sept. 1936	{ Laboratory conditions at Tinde, Tanganyika }	100-120	5	negative	43	—	0

TABLE III

Dr. Corson's observations on *T. brucei* (Strain 3)

Date	Age of strain from isolation months	Guinea-pig passages after 5th fly transmission	Pathogenicity in guinea-pigs days	Pathogenicity in mice days	Remarks
May 1936	61	25	15 [2]		These guinea-pigs were sent from Liverpool to Dr. Corson Goat 1 was inoc. from guinea- pig of 28th passage Monkey 60 was inoc. from guinea-pig of 29th passage
May-June 1936	61-62	26-28	27 [5]	12 [2]	
July-Aug. 1936	63-64	29-31	20 [5]		

TABLE IIIA

Results of Dr. Corson's attempts to transmit Strain 3 by *Glossina morsitans*

Date of attempt to transmit	Temperature at which flies were kept	No. of flies used	Infected g.-pig on which flies were fed Passage after 5th fly transmission	Result of subsequent feeds on normal animal	No. of flies alive about 40 days after 1st infecting feed	No. of flies found to be infected on dissection	
						Gut only	Gut and salivary glands
June 1936	{ Laboratory conditions at Tinde, Tanganyika }	100-120	27	negative	51	0	0
July 1936		130-160	28	"	57	0	0
Aug. 1936		100-120	Monkey 60*	"	23	0	0
Sept. 1936		200-250	" 60*	"	81	-	0

* Inoculated from guinea-pig of 29th passage of Strain 3.

TABLE V

Dr. Corson's observations on *T. brucei* (Strain 5)

Date	Age of strain from isolation months	Guinea-pig passages after 4th fly transmission	Pathogenicity in guinea-pigs days	Pathogenicity in mice days	Remarks
May 1936	61	27	28 [2]		These guinea-pigs were sent from Liverpool to Dr. Corson Monkey 57 inoc. from guinea-pig of 30th passage
May-June 1936	61-62	28-30	27 [5]	32 [2]	
July-Aug. 1936	63-64	31-33	23 [4]		

TABLE VA

Results of Dr. Corson's attempts to transmit Strain 5 by *Glossina morsitans*

Date of attempt to transmit	Temperature at which flies were kept	No. of flies used	Infected g.-pig on which flies were fed. Passage after 4th fly transmission	Result of subsequent feeds on normal animal	No. of flies alive about 40 days after 1st infecting feed	No. of flies found to be infected on dissection	
						Gut only	Gut and salivary glands
June 1936	{ Laboratory conditions at Tinde, Tanganyika }	100-120	29	negative	42	0	0
July 1936		50-60	30	"	10	0	0
July-Aug. 1936		100-120	Monkey 57*	"	60	0	0

* Inoculated from guinea-pig of 30th passage of Strain 5.

STUDIES IN CHEMOTHERAPY*

XIV.—THE STABILITY OF DRUG-RESISTANCE IN TRYPANOSOMES

BY

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AND

WARRINGTON YORKE

(Received for publication 7 December, 1936)

In this paper we record observations on the duration of the acquired character of drug-resistance in trypanosomes. Two main problems were investigated: firstly, how long resistance to various drugs persisted in trypanosomes passaged through mice by blood inoculation, and, secondly, whether the resistance survived the developmental cycle of the trypanosome in *Glossina*.

In order to answer the first of the questions, we used an old laboratory strain of *T. rhodesiense*. The history of this strain and of its resistant varieties is briefly as follows:—

Trypanosoma rhodesiense

(a) *Normal strain*. This strain was isolated from a Rhodesian case of sleeping sickness in 1923; it has since been maintained by passage through mice by blood inoculation.

(b) *Atoxyl-fast variety*. This was prepared in February-March, 1929, by the repeated administration of subcurative doses of atoxyl to mice infected with the normal strain of *T. rhodesiense*. The strain had become completely resistant to atoxyl by March 20th, 1929, in that the maximum tolerated dose of this drug failed to clear the peripheral blood of the trypanosomes. From that time up to the present, the strain has been maintained by passage through mice by blood inoculation.

(c) *Tryparsamide-fast variety*. This was prepared in February-March, 1931, by the repeated administration of subcurative doses of tryparsamide to mice infected with the normal strain. The strain was completely resistant to tryparsamide by March 10th, 1931, in that enormous doses (100 mgm. per 20 gm. mouse) of the drug failed to clear the blood of parasites. The last dose of the drug was given on March 24th, 1931. Since that time, the strain has been passaged through normal mice by blood inoculation.

(d) *Acridlavine-fast variety*. This was prepared in July-August, 1928, by the repeated administration of subcurative doses of acridlavine to mice infected with the normal strain. The strain was completely resistant on August 15th, 1928, in that the maximum tolerated doses failed to clear the blood of parasites.

*This work was assisted by a grant from the Chemotherapy Committee of the Medical Research Council.

Occasional doses of the drug were given to various of the passage-mice until August 18th, 1930. The trypanosomes always proved resistant to these doses, but whether the resistance was in any way exalted thereby it is impossible to say. Between August 18th, 1930, and October 19th, 1931, when the strain was abandoned, it was passaged through a series of 146 mice, none of which received any drug.

(e) *Bayer-fast variety*. This was prepared between May, 1931, and September, 1932, by the repeated administration of subcurative doses of Bayer 205 to mice infected with the normal strain. The difficulty experienced in making a strain resistant to Bayer is in striking contrast with the ease with which strains were made resistant to the aromatic compounds of arsenic and antimony and to acriflavine. As soon as the maximum degree of resistance was obtained on September 21st, 1932, no further doses of Bayer were administered, and the strain has since been passaged through a series of 460 mice, to none of which has any drug been given.

Table I shows the minimum effective dose (M.E.D.) and the minimum curative dose (M.C.D.) of various drugs for mice infected with the normal strain of *T. rhodesiense* and with the four resistant strains shortly after the latter were prepared. It will be noticed that the atoxyl-fast, the tryparsamide-fast and the acriflavine-fast strains were similar in the following respects: firstly, maximum tolerated doses of the aromatic compounds of arsenic and antimony and of acriflavine failed to clear the blood of infected mice; and, secondly, they were all just as sensitive to Bayer 205 as was the normal parent strain. The Bayer 205-resistant strain differed radically from the other three, in that whilst it was completely resistant to Bayer 205 it was just as sensitive to the aromatic compounds of arsenic and antimony and to acriflavine as was the normal strain.

All these strains were maintained in the laboratory by passage through mice for prolonged periods. During their maintenance their resistance to various drugs was tested from time to time. Except in the case of the Bayer 205 strain, no change in resistance was observed. The results of the last tests made in October, 1936, are shown in Table II. This Table shows that the strains made resistant to acriflavine, tryparsamide and atoxyl respectively have preserved unchanged their resistant characters during prolonged periods: e.g., the acriflavine-resistant strain for 3 years, during which time it was passaged through 337 mice; the tryparsamide-resistant strain for $5\frac{1}{2}$ years, during which it was passaged through 667 mice; and the atoxyl-resistant strain for $7\frac{1}{2}$ years, during which it was passaged through 900 mice.

In striking contrast, the Bayer 205-resistant strain, 4 years after its preparation, had completely lost all trace of resistance. The data regarding the loss of resistance of the Bayer-fast strain are interesting. When the attempt to make the strain resistant ended in September, 1932, the colossal dose of 10 mgm. per 20 gm. mouse failed to cause the trypanosomes to disappear from the blood of infected mice. Numerous tests made during 1933 showed that within a year

TABLE I
Showing the response to various drugs of mice infected with the normal strain of *T. rhodesiense* and of the resistant strains immediately after they were prepared

Strain	Date when resistant strain was prepared	Response of infected mice to the various drugs					
		Tryparsamide		Halarsol		Acriflavine	
		mgm. per 20 gm. mouse	M.E.D.	mgm. per 20 gm. mouse	M.E.D.	mgm. per 20 gm. mouse	M.E.D.
Normal							
Atoxyl-fast	Mar. 1929	6.0	20.0	0.02	0.1	0.4	4.0
Tryparsamide-fast	Mar. 1931	>50.0		>0.64	>0.4		15.0
Acriflavine-fast	Aug. 1928	>50.0		>0.64	>0.4		15.0
Bayer 205-fast	Sept. 1932	>50.0		>0.64	>0.4		15.0
				0.02	0.1	4.0	4.0
							0.025
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the strain had lost a great deal of its resistance. Of 22 mice treated with a dose of 1 mgm. per 20 gm. mouse, 21 became negative, and all but 4 of these relapsed within 15 days; of 23 mice treated with 0.5 mgm. per 20 gm. mouse, 15 became negative, and all but 2 relapsed within 8 days; and of 5 mice treated with 0.25 mgm. per 20 gm. mouse, none became negative. It appears, therefore, that the resistance of the strain had so far decreased that the minimum effective dose of the drug for infected mice had fallen from something greater than 10 mgm. per 20 gm. mouse to between 0.5 and 1.0 mgm. per 20 gm. mouse. Unfortunately, the strain was not examined again for about 3 years, when (in September, 1936) it was found, as shown in Table II, to have lost all trace of resistance and to be just as sensitive to the drug as was the normal parent strain.

We conclude from this work that resistance to acriflavine and to the aromatic compounds of arsenic and antimony is rapidly acquired and persists indefinitely, or at least for a period of $7\frac{1}{2}$ years, when the trypanosome is passaged through normal mice by blood inoculation. The resistance to Bayer 205 is, however, slowly acquired, and is gradually lost when the trypanosome is passaged through normal mice by blood inoculation.

In order to ascertain whether a trypanosome retains its acquired character of drug-resistance during its developmental cycle in the invertebrate host—the tsetse-fly—we used a recently isolated strain of *T. brucei*, to which reference has already been made in previous papers of this series.

T. brucei

(a) *Normal strain.* This is Strain 3 of our previous paper (Murgatroyd and Yorke, 1937). It originated from wild *G. morsitans* in April, 1931, and has since been maintained by passage through guinea-pigs, partly by blood inoculation and partly by transmission through *G. morsitans*.

(b) *Tryparsamide-resistant variety.* This is Strain 5 of our previous paper (Murgatroyd and Yorke, 1937). It originated in September, 1932, by the repeated administration of subcurative doses of tryparsamide to a guinea-pig of the 11th passage of the normal strain (Yorke, Murgatroyd and Hawking, 1933). It has since been maintained in the same manner as the normal strain, viz., by passage through a series of guinea-pigs, partly by means of blood inoculation and partly by transmission through *G. morsitans*. Since the trypanosome was made resistant to tryparsamide in September, 1932, it has not been subjected to the action of any drug.

(c) *Tryparsamide-Bayer-resistant variety.* This originated in February, 1933, from the tryparsamide-resistant strain mentioned above, after it had passed twice through *G. morsitans*. A series of guinea-pigs was treated with gradually increasing subcurative doses of Bayer 205 between February, 1933, and January, 1934. The strain was then transmitted through guinea-pigs by blood inoculation until September, 1934, when it was again passed through *G. morsitans*.

Details of the history of these three strains are given in the plan.

Information regarding the response to various drugs of mice infected with the normal strain of *T. brucei* and of the tryparsamide-resistant variety at different stages of their life has been given in Studies X and XIII of this series (Yorke, Murgatroyd and Hawking, 1933; Murgatroyd and Yorke, 1937). Consequently, all that need have been said is that, as is shown in Table III, the tryparsamide-resistant variety has not lost any of its resistance after 4 years' maintenance in the laboratory, during which time it has passed through 59

TABLE III

Comparing the response to arsenicals of mice infected with the normal strain of *T. brucei* and of its tryparsamide-resistant variety when they were first obtained and after prolonged maintenance in the laboratory

Strain	Date examined		No. of passages between first and last test	Response of infected mice to			
				Tryparsamide mgm. per 20 gm. mouse		Halarsol mgm. per 20 gm. mouse	
				M.E.D.	M.C.D.	M.E.D.	M.C.D.
Normal	When first examined	Mar.-June 1932		8.0	40.0	0.03	>0.32
	4½ years later	Oct. 1936	67 guinea-pigs (5 times transmitted by <i>G. morsitans</i>)			0.02	0.16
Tryparsamide-resistant variety	When first prepared	Sept. 1932		>50.0		>0.64	
	4 years later	Oct. 1936	59 guinea-pigs (4 times transmitted by <i>G. morsitans</i>)	>50.0		>0.64	

guinea-pigs and has, on four occasions, been cyclically transmitted by *G. morsitans*.

As will be seen from the plan, the attempt to make the tryparsamide-fast strain also resistant to Bayer 205 began in February, 1933, by the administration of frequent subcurative doses of the drug to infected guinea-pigs. With the exception of a break between June and August, 1933, the work continued until January, 1934. Before the strain was made Bayer-resistant, the minimum effective dose of the drug for infected guinea-pigs was 1.0 mgm. per kilo., this

amount sufficing to clear the blood of infected animals for from 2 to 12 days. When the attempt to produce resistance ended in January, 1934, such large doses as 20 and 30 mgm. failed to cause the trypanosomes to disappear from the blood of the infected guinea-pigs. The strain was then passaged by blood inoculation through a series of 6 guinea-pigs until September, 1934, when it was passed through *G. morsitans* and thence to other guinea-pigs. The resistance of the strain to Bayer 205 was tested in guinea-pigs before and after transmission by *Glossina*, with the results shown in Table IV.

TABLE IV

Showing the response to Bayer 205 of guinea-pigs infected with the tryparsamide-fast strain of *T. brucei* before and after it was made Bayer-fast and after the Bayer-fast strain was passed through *G. morsitans*

Strain	Date examined		Response of infected guinea-pigs to Bayer 205. Minimum effective dose mgm. per kilo.
Tryparsamide-fast	Before making Bayer-fast	Jan. 1933	1.0
Tryparsamide-Bayer-fast	Before transmission by <i>Glossina</i>	Sept. 1934	10.0-20.0
	After transmission by <i>Glossina</i>	Oct. 1934	10.0

This Table shows that Bayer-resistance survives at least one developmental cycle of the trypanosome in *G. morsitans*.

SUMMARY

Resistance to the aromatic compounds of arsenic and antimony and to acriflavine is rapidly acquired by trypanosomes and persists indefinitely. A strain of *T. rhodesiense* made resistant to atoxyl in 1929, and subsequently passaged through mice by blood inoculation, has maintained unimpaired its resistant character for a period of 7½ years, during which the strain has passed through a series of 900 mice. Strains of *T. rhodesiense* made resistant to tryparsamide and to acriflavine respectively behaved similarly.

Resistance to the aromatic arsenicals likewise survives unimpaired when the trypanosome is passed repeatedly through the natural intermediate host, *Glossina*. A strain of *T. brucei* made resistant to tryparsamide in 1932, and subsequently transmitted through guinea-pigs, partly by blood inoculation and partly by the tsetse-fly, has maintained unimpaired its resistant character for a period of 4 years, during which it has passed through 59 guinea-pigs and 4 times through *G. morsitans*.

Resistance to Bayer 205 is developed very slowly and is gradually lost. A strain of *T. rhodesiense* was made resistant to this drug by repeated administration of subcurative doses to infected mice between May, 1931, and September, 1932. The resistant strain was then passaged through mice by blood inoculation. Within a year the resistant strain had lost at least 90 per cent. of its resistance, and within 4 years had lost all trace of resistance.

Resistance to Bayer 205 survives at least one passage through *G. morsitans*. A trypanamide-fast strain of *T. brucei* was made also resistant to Bayer 205 by the repeated administration of subcurative doses of the drug to a series of infected guinea-pigs over a period of a year. After the resistant strain had been passaged through a short series of guinea-pigs it was passed through *G. morsitans*. Examination of the strain before and after transmission by *G. morsitans* showed that its Bayer 205-resistant character had survived the cyclical development of the trypanosome in the tsetse-fly.

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STUDIES IN CHEMOTHERAPY*

XV.—OBSERVATIONS ON THE LOSS OF TRANSMISSIBILITY BY *GLOSSINA MORSITANS* OF *T. BRUCEI* MAINTAINED IN A EUROPEAN LABORATORY

BY

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AND

WARRINGTON YORKE

(Received for publication 10 January, 1937)

In a previous paper we have shown that a strain of *T. brucei*—both normal and arsenic-resistant varieties—maintained in the laboratory by passage through guinea-pigs, partly by blood inoculation and partly by *Glossina morsitans*, eventually lost its capacity for being transmitted by the fly. In the present paper we propose to review these observations, with a view to considering whether they throw any light on the mechanism whereby a strain of trypanosomes becomes non-transmissible by *Glossina*.

The flies used in these experiments were sent to us in the pupal state by air-mail from Tanganyika, and we are much indebted to Dr. J. F. Corson for his kindness in this matter. They were fed on the infected animals on either two or three occasions, with 48 to 72 hours' starvation between each feed. In those cases where there were three infective feeds, they were, as a rule, on the 1st, 4th and 7th days, and in those in which there were only two the infective feeds were on the 1st and 4th days. When there were three infective feeds, the first feed on the normal animal was on the 10th day, and when there were only two infective feeds the first normal feed was on the 7th day. During the night, the flies were always kept in an incubator at a temperature which was usually between 82° F. and 86° F., and in a more or less saturated atmosphere. During the daytime, when the weather was at all suitable, they were kept in a heated glass-house, the temperature of which varied between 60° F. and 98° F. On some occasions, however, when the weather was not suitable, they were kept during the daytime in an incubator, at temperatures which in most cases varied between 90° F. and 96° F., in a moist atmosphere.

The artificial conditions under which the flies were kept resulted in a very considerable mortality, especially during the early days of the experiments; and in recording our results we have neglected all those which died before the 11th day of the experiment, i.e., before they had fed on a clean animal, and in less than 4 or 7 days after the last infecting feed. All flies which died on the 11th day and later were carefully dissected and the various organs (salivary glands, proventriculus and gut) examined for trypanosomes.

*This work was assisted by a grant from the Chemotherapy Committee of the Medical Research Council.

By this means we were able to exclude all flies having a temporary infection in the lumen of the gut, resulting from trypanosomes merely retained from a previous infecting feed, and to confine our attention to those flies in which the infection had actually established itself outside the peritrophic membrane.

In Tables I and IA to III and IIIA, we give details of the results of successive attempts to infect *G. morsitans* with the collateral branches of *T. brucei*, viz., Strain 3 (normal branch), Strain 5 (tryparsamide-resistant branch) and Strain 6 (the tryparsamide-resistant branch made also resistant to Bayer 205). As will be

TABLE I

Showing passage of Strain 3 (*T. brucei*, normal branch) through guinea-pigs, partly by blood inoculation and partly by *G. morsitans*

Date	Age of strain from isolation months	Passage	Attempt to transmit by <i>G. morsitans</i>
Apr. 1931	0	Wild <i>G. morsitans</i> Dog	
May 1931-Aug. 1932	1-16	G.-pig 1-10	
Sept. 1932	17	" 11*	
Sept. 1932	17	" 12	
Oct. 1932	18	<i>G. morsitans</i>	1st attempt successful
Nov. 1932-July 1933	19-27	G.-pig 1-9	
Aug. 1933	28	" 10	
Sept. 1933	29	<i>G. morsitans</i>	2nd " "
Oct. 1933	30	G.-pig 1	
Nov. 1933	31	<i>G. morsitans</i>	3rd " "
Nov. 1933-Aug. 1934	31-40	G.-pig 1-9	
Sept. 1934	41	" 10	
Sept. 1934	41	<i>G. morsitans</i>	4th " "
Oct. 1934-Jan. 1935	42-45	G.-pig 1-5	
Feb. 1935	46	" 6	
Mar. 1935	47	<i>G. morsitans</i>	5th " "
Apr.-Nov. 1935	48-55	G.-pig 1-12	
Dec. 1935	56	" 13	6th " unsuccessful
Jan.-Mar. 1936	57-59	" 14-20	
Apr. 1936	60	" 21-22	7th " "
May-June 1936	61-62	" 23-26	8th " "
[June 1936	62	" 27	9th " "]†
June-July 1936	62-63	" 27-29	10th " " "
[July 1936	63	" 28	11th " "]†
July-Aug. 1936	63-64	" 30-31	12th " " "
[Aug. 1936	64	" 29	13th " "]†
Sept.-Oct. 1936	65-66	" 32-33	14th " " "
[Sept. 1936	65	" 32	15th " "]†

* From this guinea-pig Strain 5 originated, *vide* Table II.

These attempts to transmit were carried out by Dr. Corson in Tanganyika.

TABLE IA
Results of successive attempts to transmit Strain 3 by *Glossina morsitans*

Date of attempt to transmit	No. of attempt to transmit	Temperature at which flies were kept	No. of flies used	No. of flies alive 11 days after 1st infecting feed	No. of flies found to be infected on dissection 11 days or more after 1st infecting feed		
					Gut only	Gut and proventriculus	Gut, proventriculus and salivary glands
Sept. 1932	1	{ Day 60°-80° F. Night 82°-86° F.	89	59	1 12th day	16th day 19th " 7 21st " 23rd " 39th " 54th " [2]	2 1 fly proved infective in 11 days
Sept. 1933	2	{ Day 96° F. Night 82°-84° F.	213	58	1 20th day	12th day 22nd " 26th " 10 30th " [2] 34th " 36 h " 39th " [2] 40th "	1 This became infective on approx. 21st day
Nov. 1933	3	{ Day 60°-75° F. Night 82°-84° F.	90	41	2 11th day 38th "	16th day 8 21st " 37th " 38th " [5]	1 Infective about 21st day
Sept. 1934	4	{ Day 60°-80° F. Night 82°-86° F.	179	79	0	4 24th day 25th " [3]	4 Infective about 12th day

TABLE IA—(Continued)
Results of successive attempts to transmit Strain 3 by *Glossina morsitans*

Date of attempt to transmit	No. of attempt to transmit	Temperature at which flies were kept	No. of flies used	No. of flies alive 11 days after 1st infecting feed	No. of flies found to be infected on dissection 11 days or more after 1st infecting feed		
					Gut only	Gut and proventriculus	Gut, proventriculus and salivary glands
Mar. 1935	5	{ Day 60°-80° F. Night 82°-84° F.	145	89	0	23rd day [2] 8 24th " [3] 25th " 42nd " 48th "	1
Jan. 1936	6	{ Day 60°-80° F. Night 80°-82° F.	237	150	0	11th day 16th " 20th " 21st " 22nd " 28th " 19 30th " 31st " 33rd " 34th " [2] 35th " [2] 41st " [4] 44th " 46th "	0
Mar. 1936	7	{ Day 70°-86° F. Night 82°-86° F.	277	171	3 11th day 15th " 20th "	14th day 17th " 23rd " 24th " [2] 27th " 15 30th " [2] 31st " 33rd " 34th " [2] 39th " [2] 50th "	0

TABLE 1A—(Continued)
Results of successive attempts to transmit Strain 3 by *Glossina morsitans*

Date of attempt to transmit	No. of attempt to transmit	Temperature at which flies were kept	No. of flies used	No. of flies alive 11 days after 1st infecting feed	No. of flies found to be infected on dissection 11 days or more after 1st infecting feed		
					Gut only	Gut and proventriculus	Gut, proventriculus and salivary glands
June 1936	8	{ Day 80°-98° F. Night 86° F.	287	58	0	0	0
[June 1936	9	Laboratory conditions at Tinde, Tanganyika	100-120	51	0		0]†
July 1936	10	{ Day 82°-88° F. Night 82°-88° F.	615	435	14th day 3 21st 22nd	0	0
[July 1936	11	Laboratory conditions at Tinde, Tanganyika	130-160	57			0]†
Aug. 1936	12	{ Day 60°-90° F. Night 77° F.	163	130	0	0	0
[Aug. 1936	13	Laboratory conditions at Tinde, Tanganyika	100-120	23			0]†
Sept. 1936	14	{ Day 82°-86° F. Night 82°-86° F.	255	64	0	0	0
[Sept. 1936	15	Laboratory conditions at Tinde, Tanganyika	200-250	81			0]†

† These attempts to transmit were carried out by Dr. Corson in Tanganyika.

seen in Tables I, II and III, the strains were in the intervals between the fly transmissions passed from guinea-pig to guinea-pig by means of blood inoculation.

The Tables show that, although the opportunities to attempt transmission by *G. morsitans* were relatively few, with the result that the fly passages were often separated from one another by many months, during which the strains

TABLE II

Showing passage of Strain 5 (*T. brucei*, trypanamide-resistant branch) through guinea-pigs, partly by blood inoculation and partly by *G. morsitans*

Date	Age of strain from isolation months	Passage	Attempt to transmit by <i>G. morsitans</i>
Sept. 1932	17	G.-pig 11*	
Nov. 1932	19	<i>G. morsitans</i>	1st attempt successful
Nov. 1932	19	G.-pig 1	
Dec. 1932	20	<i>G. morsitans</i>	2nd " "
Dec. 1932–Nov. 1933	20–31	G.-pig 1†–12	
Dec. 1933	32	" 13	
Jan. 1934	33	<i>G. morsitans</i>	3rd " "
Jan.–Sept. 1934	33–41	G.-pig 1–7	
Oct. 1934	42	" 8	
Oct. 1934	42	<i>G. morsitans</i>	4th " "
Nov. 1934–Dec. 1935	43–56	G.-pig 1–18	
Jan. 1936	57	" 19	5th " unsuccessful
Feb.–April 1936	58–60	" 20–25	
May 1936	61	" 26	6th " "
[June 1936	62	" 29	7th " "]†
[July 1936	63	" 30	8th " "]†
[July–Aug. 1936	63–64	" 30	9th " "]†

* This guinea-pig, which represents the starting-point of Strain 5, is the guinea-pig of the 11th passage of Strain 3; it was given numerous subcurative doses of trypanamide in order to make the infection trypanamide-resistant.

† From this guinea-pig Strain 6 originated, *vide* Table II.

‡ These attempts to transmit were carried out by Dr. Corson in Tanganyika.

had to be passed through a considerable series of guinea-pigs by blood inoculation, all three strains retained their transmissibility for prolonged periods—Strain 3 (normal) for at least 47 months, Strain 5 (trypanamide-fast) for at least 42 months, and Strain 6 (trypanamide-Bayer-fast) for at least 41 months.

Numerous subsequent attempts, however, to transmit Strain 3 (normal) and Strain 5 (trypanamide-resistant) failed. In the case of Strain 3, six unsuccessful attempts to transmit by *G. morsitans* were made by us in Liverpool

TABLE IIA
Results of successive attempts to transmit Strain 5 (*T. brucei*, trypanamide-resistant branch) by *Glossina morsitans*

Date of attempt to transmit	No. of attempt to transmit	Temperature at which flies were kept	No. of flies used	No. of flies alive 11 days after 1st infecting feed	No. of flies found to be infected on dissection 11 days or more after 1st infecting feed		
					Gut only	Gut and proventriculus	Gut, proventriculus and salivary glands
Nov. 1932	1	{ Day 90° F. Night 82°-86° F.	160	49	0	0	1 Infection not more than 23 days
Dec. 1932	2	{ Day 90° F. Night 82°-86° F.	168	109	1 40th day	38th day 3 43rd " 46th "	3 Infection between 19th and 26th days
Jan. 1934	3	{ Day 96° F. Night 82°-84° F.	186	88	1 26th day	18th day 21st " 22nd " 24th " 26th " [2] 51st " 60th "	1
Oct. 1934	4	{ Day 60°-80° F. Night 82°-86° F.	257	153	2 16th day 17th "	10th day [3] 22nd " 25th " 33rd " [2] 38th " [3]	2 Infection about 14 days

TABLE IIA—(Continued)

Results of successive attempts to transmit Strain 5 (*T. brucei*, trypanamide-resistant branch) by *Glossina morsitans*

Date of attempt to transmit	No. of attempt to transmit	Temperature at which flies were kept	No. of flies used	No. of flies alive 11 days after 1st infecting feed	No. of flies found to be infected on dissection 11 days or more after 1st infecting feed		
					Gut only	Gut and proventriculus	Gut, proventriculus and salivary glands
Jan. 1936	5	{ Day 60°-80° F. Night 80°-82° F.	171	113	0	0	0
May 1936	6	{ Day 70°-86° F. Night 82°-86° F.	285	182	0	0	0
[June 1936	7	Laboratory conditions at Tinde, Tanganyika	110	42	0		0]†
[July 1936	8	" "	55	10	0		0]†
[July-Aug. 1936	9	" "	110	60			0]†

† These attempts to transmit were carried out by Dr. Corson in Tanganyika.

between January and October, 1936, and four unsuccessful attempts were made by Corson in Tanganyika between June and September, 1936; and in the case of Strain 5 two unsuccessful attempts to transmit were made by us in Liverpool between January and May, 1936, and three unsuccessful attempts by Corson in Tanganyika between June and August, 1936. In Table IV we summarize the total results of all the experiments made with the two strains whilst they were still transmissible, i.e., up to March, 1935; and the total results of the experiments made subsequent to January, 1936, when we were unable to

TABLE III

Showing passage of Strain 6 (*T. brucei*, tryparsamide-Bayer-resistant branch) through guinea-pigs, partly by blood inoculation and partly by *Glossina morsitans*

Date	Age of strain from isolation months	Passage	Attempt to transmit by <i>G. morsitans</i>
Jan. 1933	21	G.-pig 1*	1st attempt successful
Apr. 1933	24	" 2†	
July 1933	27	" 3	
Aug. 1933	28	" 4	
Aug. 1933	28	<i>G. morsitans</i>	
Sept. 1933	29	G.-pig 1†	
Dec. 1933	32	" 2	
Jan. 1934	33	" 3†	
Feb. 1934	34	" 4†	
Apr.-Sept. 1934	36-41	" 5-10	
Sept. 1934	41	<i>G. morsitans</i>	2nd " "
Oct. 1934	42	G.-pig 1-2	

* This guinea-pig, which represents the starting-point of Strain 6, is a guinea-pig of Strain 5 after it had twice passed through *G. morsitans* (Table II); it was given numerous subcurative doses of Bayer 205.

† These guinea-pigs were also given numerous subcurative doses of Bayer 205 in order to make the strain Bayer-resistant.

transmit. The Table shows the total number of flies used in these experiments and the number of salivary gland infections discovered. From the Table it is seen that in the experiments conducted up to March, 1935, 1,893 flies were used and 26 salivary gland infections obtained; whereas in those conducted subsequent to January, 1936, 3,155 flies were used and not a single salivary gland infection was found on dissection, nor was the infection transmitted to any of the normal animals on which these flies were fed. These figures appear to us to afford exceedingly strong evidence that the strains had actually become non-transmissible.

TABLE IIIA
Results of successive attempts to transmit Strain 6 by *Glossina morsitans*

Date of attempt to transmit	No. of attempt to transmit	Temperature at which flies were kept	No. of flies used	No. of flies alive 11 days after 1st infecting feed	No. of flies found to be infected on dissection 11 days or more after 1st infecting feed		
					Gut only	Gut and proventriculus	Gut, proventriculus and salivary glands
Sept. 1933	1	{ Day 96° F. Night 82°-84° F.	278	67	11th day 4 13th " 18th " 22nd "	16th day	8
						23rd " 24th " [2] 25th " [2] 26th " 28th " 30th " 35th " [2]	
Sept. 1934	2	{ Day 60°-80° F. Night 82°-86° F.	128	52	0	13th day	2
						15th " 19th " 26th " 33rd "	

TABLE IV

Comparing the results of the transmission experiments conducted up to March, 1935, with those of similar experiments conducted subsequent to January, 1936

Strain	Transmission experiments prior to March, 1935, when strains were transmissible				Transmission experiments subsequent to January, 1936, when attempts to transmit were unsuccessful		
	No. of flies used	No. of flies alive on 11th day after 1st infected meal	No. of salivary gland infections discovered	No. of flies used	No. of flies alive on 11th day after 1st infected meal	No. of salivary gland infections discovered	Remarks
Strain 3 (normal)	716	326	9	1,834 590*	1,008 212**	0 0	* Corson's experiments in Tanganyika ** No. of flies alive on 30th day after 1st infected meal
Strain 5 (tryparsamide-resistant)	771	399	7	456 275*	295 112**	0 0	* Corson's experiments in Tanganyika ** No. of flies alive on 30th day after 1st infected meal
Strain 6 (tryparsamide-Bayer-resistant)	406	119	10				
	1,893	844	26	3,155	1,627	0	

Having thus reached the general conclusion that the trypanosome (both normal and drug-resistant strains) remained transmissible by *G. morsitans* for at least 4 years after isolation, and that it subsequently became non-transmissible, we can now pass to a more detailed examination of the results obtained in the successive attempts at transmission, with a view to ascertaining whether they provide any information regarding the process by which the trypanosome became non-transmissible by the tsetse.

If we consider the results of dissection of the flies which lived longer than 10 days after the first infecting feed (Tables IA, IIA and IIIA), we cannot but be impressed by the fact that so long as the strains remained transmissible, and consequently salivary gland infections were obtained, there was always a very large number of flies which exhibited 'gut' or 'gut + proventriculus' infection. In the experiments conducted between June and September, 1936, when the trypanosome had become non-transmissible, no salivary gland infections were found; there were also no 'gut + proventriculus' infections, and very few 'gut only' infections. At the beginning, therefore, when the trypanosome is readily transmissible, we find numerous flies with heavy infections of the gut and proventriculus, whilst at the end of the experiment, when the trypanosome is definitely non-transmissible, we find no 'gut + proventriculus' infections and practically no 'gut only' infections. This suggests that in the final stages the trypanosome had completely lost the capacity of establishing itself in the tsetse. What, however, happened between these two stages of capacity on the part of the trypanosome to complete its development in *Glossina* on the one hand, and its inability to infect the fly at all on the other hand? Interesting information on this matter is apparently supplied by the experiments conducted with Strain 3 in January and March, 1936 (Table IA). Here we see evidence of an intermediate stage in which numerous 'gut + proventriculus' infections were found, but no salivary gland infections. This stage was missed in the case of Strain 5, possibly owing to the very long interval (15 months) which elapsed between the last successful transmission (October, 1934) and the next attempt to transmit (January, 1936). One interpretation of these results is obviously that the trypanosome first lost its power of completing its developmental cycle in the tsetse by invading the salivary glands, although it still retained its capacity of reaching the proventriculus. Before, however, we should be justified in reaching such a conclusion we must satisfy ourselves that these proventriculus infections do not merely represent immature infections, which, had the flies been kept longer under suitable conditions, would eventually have resulted in mature salivary gland infections.

Unfortunately, the state of the proventriculus has been almost entirely ignored by those who have worked on the development of the *brucei* group of trypanosomes in *Glossina*. The only published data bearing on this matter seem to be those supplied by Taylor (1932) and by van Hoof and Henrard (1934). In a Table summarizing the infection rates obtained in *G. tachinoides*

with West African strains of *T. gambiense*, Taylor gives information regarding the number of 'gut only' infections, 'gut + proventriculus' infections, and 'total' infections, found on dissecting the flies at various periods between the 11th and 30th day (in the great majority of instances between the 15th and 20th day) after the infecting meal, in respect of each of the 26 strains examined by him. Taylor concludes from this work that 'Once the extra-peritrophic space is infected development proceeds uninterruptedly and the infection normally persists until the death of the fly.' Whilst he does not say in so many words that flies with a proventriculus infection will necessarily eventually develop a salivary gland infection, provided that they live long enough, this seems to be clearly implied. Taylor lays great stress on the importance of examining the proventriculus because 'it provides valuable additional means of detecting maturing infections and is never involved in transitory gut infections.' Furthermore, in assessing the cyclical developmental rate, Taylor includes the 'gut + proventriculus' infections with the 'total' infections.

When we examine the evidence upon which Taylor bases his conclusions we find that it is not entirely adequate. In his transmission experiments conducted under normal laboratory conditions, we see that, as a result of his dissections of flies 11 to 30 days (for the most part under 22 days) after the infecting meal, 221 were found to harbour trypanosomes—11 'gut only,' 155 'gut + proventriculus,' and 55 'gut + proventriculus + salivary glands' infections. Thus, in only 33 (26 per cent.) of the 210 flies in which the proventriculus was involved had the infection reached the salivary glands. Taylor's view is apparently that, had the dissections been made at a later date, all the flies with proventriculus infections would also exhibit salivary gland infections. The data presented, however, supply no justification for this view; and neither do the observations recorded in Taylor's second group of experiments. In this group of experiments the flies were kept at a temperature of 37° C. for 16 to 24 hours per day during the 4 days' infecting meals, instead of at laboratory temperature, as in the first group of experiments. Taylor claims that by this device 'total' infection rates of from 10.5 to 77.8 per cent. were obtained, whereas the corresponding infection rates in control series kept at room-temperature were from zero to 10.5 per cent. Assuming the truth of this contention—and the figures are very convincing—the later group of experiments no more justifies Taylor's statement that proventriculus infection implies eventual salivary gland infection than does his first group of experiments. So far as it is possible to judge from the information supplied, the majority of the dissections in the second group of experiments were performed about the same time after the infecting meals as were those in the first group of experiments, viz., within 22 days. In all, 482 were found to harbour trypanosomes—61 'gut only,' 292 'gut + proventriculus,' and 129 'gut + proventriculus + salivary glands' infections. Thus in only 129 (30.6 per cent.) of the 421 flies in which the proventriculus was involved did the infection reach the salivary glands. Although, therefore, increasing the

temperature at which the flies were maintained during the 4 days' infecting meals may have increased the infection rate, it failed to alter materially the proportion of salivary gland infections to proventriculus infections; in the first case this was 26.2 per cent., and in the second 30.6 per cent.

From this analysis of Taylor's data we can only conclude that, although he may be correct when he lays down that proventriculus infection necessarily implies eventual salivary gland infection, he has produced no adequate evidence to support his contention.

In contradistinction to Taylor, Duke (1933*b*) holds 'that in all but exceptionally transmissible strains there will always be a certain number of infected flies in which, no matter how long they live, the glands never will become infected.' In support of this contention, Duke has assembled a vast amount of information regarding his findings in infected flies dissected more than 30 days after the infecting meal. The interval between the infecting meal and dissection is, of course, of great importance in attempting to solve the problem under consideration, and Duke chooses 30 days or over, because he believes that if the salivary glands are going to become infected they will do so in less than 30 days after the infecting meal. He has collected information on this subject from the observations made by himself and various other workers, viz., Kleine and Fischer (1913), Kinghorn, Yorke and Lloyd (1913), Bruce *et al.* (1915), Lloyd (1930) and Taylor (1932). From his analysis of the data Duke concludes that 'It is very exceptional for the biological cycle of man's trypanosomes in *Glossina* to take more than 30 days for its completion. As a general rule, 25 days or less are required.'

Unfortunately, Duke did not trouble himself about the state of the proventriculus, and contents himself with recording whether he encountered 'gut only' infections or 'salivary gland' (total) infections. We find, however, that Duke produces overwhelming evidence that *G. palpalis* or *G. morsitans* dissected over 30 days after the infecting meal can harbour trypanosomes in the gut without the infection having extended to the salivary glands. Analysis of Duke's figures shows that, whereas the great majority of his flies were dissected between the 31st and 40th day after the infecting meal, no less than 48 were dissected between the 41st and 50th day, and 7 between the 51st and 60th day.

Van Hoof and Henrard (1934) give valuable information regarding their findings on dissection of *G. palpalis* used by them in a long series of attempts to transmit *T. gambiense* at Leopoldville. In all but two of their 16 experiments the state of the gut, proventriculus and salivary glands is recorded. Neglecting the two experiments in which the proventriculus was not examined, we find that they discovered 34 'salivary gland' infections, 45 'gut + proventriculus' infections, and 150 'gut only' infections. Although it was impossible to ascertain the exact time at which the salivary glands became infected in each of the 34 occasions on which this happened, yet, on the assumption that the incubation period of the infection in the guinea-pig is 10 to 12 days, van Hoof and Henrard

are able to assess the approximate date on which the first fly of each experiment became infective and, therefore, on which the developmental cycle was complete. In most of the experiments the cycle was completed within 17–32 days, but in a few it was not complete until much later (48–80 days).

The explanation of the retarded development which was occasionally encountered by van Hoof and Henrard is not clear, but possibly it is in part at least related to differences in climatic conditions. Long ago, Kinghorn and Yorke (1912) drew attention to the fact that, whereas it was quite easy to transmit *T. rhodesiense* by *G. morsitans* in the Luangwa Valley, it was impossible to do so at Ngoa on the Congo-Zambesi watershed. When, however, the flies were kept in the incubator at about 85° F., instead of in the laboratory at 60°–65° F., transmission experiments succeeded at the latter place, the duration of the developmental cycle in the fly being from 13 to 21 days.

In other experiments at Ngoa it was shown that, when *G. morsitans*, which had been fed on animals infected with *T. rhodesiense* 40 to 61 days previously, and were still incapable of transmitting the infection, were placed in the incubator at 85° F. for a few days, a number of them became infective and on dissection showed invasion of the salivary glands. From this work Kinghorn and Yorke concluded that the earlier stages of the development of the parasite in the fly can occur at comparatively low temperatures (60° F.), and that trypanosomes can persist at this stage for at least 60 days. For the completion of the cycle, it is necessary for the temperature to which the flies are subjected to be raised to a considerable extent (75°–85° F.). It is interesting to note that the experiments in which van Hoof and Henrard (1934) obtained the most protracted developmental cycles were performed during the cold season of the year.

Whilst, therefore, Duke's dictum that 'It is very exceptional for the biological cycle of man's trypanosomes in *Glossina* to take more than 30 days for its completion' is probably correct, yet there seems little doubt that, in certain places and under certain conditions, the cycle may be much retarded: and probably low temperature is the most important factor responsible for bringing this about.

If we now return to a consideration of the present observations, it will be seen from Tables IA, IIA and IIIA that 2,147 of the flies used in the transmission experiments performed in Liverpool lived longer than 10 days after the first infective meal; these flies were dissected at various times after this period, and their organs (salivary glands, proventriculus and gut) examined for trypanosomes. All flies dying in less than 11 days (i.e., 4 or 7 days after the last infecting meal) were ignored, so that, as stated earlier, we were able to confine our attention to infections which had definitely established themselves outside the peritrophic membrane. In all, 152 flies were found to be infected; in 18 (12 per cent.) the trypanosomes were limited to the gut outside the peritrophic membrane, in 108 (71 per cent.) both gut and proventriculus were invaded, and in 26 (17 per cent.) the infection was 'total,' the trypanosomes having reached the salivary glands.

The fact that in only 26 of the 134 flies in which the trypanosomes reached the proventriculus did the infection extend still further forward to the salivary glands bears upon the important question to which reference has already been made, viz., had all the flies lived long enough, would the infection have always extended to the salivary glands, and the flies become infective?

Our observations appear to throw some light upon the problem. In 9 of the 26 flies in which the salivary glands were invaded, it was possible from feeding experiments on normal animals to assess approximately the date on which the flies became infective, and hence the date on which the cycle of development was complete. Five of these 9 flies infected normal animals between the 10th and 15th days after their first infecting meal, and the remaining 4 were found to be infective between the 19th and 26th day. These results agree substantially

TABLE V

Showing the location of trypanosomes in flies found to be infected at various intervals after the 1st infecting feed

Days after 1st infecting feed	Site of infection		
	Gut only	Gut + proventriculus	Gut + proventriculus + salivary glands
11-15	7	8	Of the 25 flies which on dissection showed invasion of the salivary glands, it was possible by feeding experiments to obtain an approximate date by which the salivary glands had become infected in 9 cases. In 5 of these the flies had become infective between the 10th and 15th day, and in 4 between the 19th and 26th day
16-20	5	9	
21-25	3	29	
26-30	1	14	
31-35	0	16	
36-40	2	17	
41-45		7	
46-50		4	
51-55		3	
56-60		1	

with those of previous workers, and are in conformity with Duke's generalization that if a fly is going to become infective it will have done so by the 30th day.

Reference to Table V shows that in a considerable number of flies dissected later than 25 days after the first infecting meal the trypanosomes were limited to the gut and proventriculus and the salivary glands were free. Thus, in the 35 infected flies dissected between 31 and 40 days after the first infecting meal the trypanosomes were limited to the gut and proventriculus, and the same applies to the 11 flies dissected between the 41st and 50th days, and to the 4 dissected between the 51st and 60th days after the infecting meal.

These observations confirm and extend those of Duke, who, as already mentioned, found that tsetse can harbour trypanosomes in the gut for long

beyond 30 days without the infection spreading to the salivary glands. Unfortunately, as we have previously pointed out, Duke did not examine the state of the proventriculus, but judging from our own observations it seems reasonable to assume that had the matter been investigated Duke would have found that a very large proportion of his late 'gut' infections were in reality 'gut + proventriculus' infections. But be that as it may, our records suffice to show that, under conditions which permit of the completion of the development cycle in certain flies within 25 days, other flies can harbour trypanosomes in the proventriculus (and gut) for as long as 60 days without the infection spreading to the salivary glands. This observation seems to afford strong support for the view that proventricular infection does not necessarily mean eventual gland infection.

The very large proportion of the infected flies showing involvement of the proventriculus is interesting, and raises the question whether this organ does not always become infected in those flies in which the infection establishes itself outside the peritrophic membrane at the hinder end of the gut, provided that the flies live sufficiently long. In order to examine this and other questions more closely, we show in Table V the situation of the trypanosomes found in the infected flies dissected during each 5-day period beyond the initial 10 days after the first infecting meal.

In the flies dissected between the 11th and 15th days after the first infecting meal, 'gut only' infections were about as common as 'gut + proventriculus' infections, i.e., 7 'gut only' infections, as compared with 8 'gut + proventriculus' infections. In the flies dissected between the 16th and 20th days, the proportion of 'gut only' to 'gut + proventriculus' infections fell from 5 to 9, and in the 21st to 25th day period from 3 to 29. In flies which lived beyond the 25th day, 'gut only' infections were very rarely encountered. The earlier, then, the dissections were made, the higher the proportion of 'gut only' infections encountered; and this, in conjunction with the fact that the earliest proventriculus infections found by us were on the 9th or 10th day, raises the question whether many or all of the 'gut only' infections would not, had the flies lived longer, eventually have extended forward to the proventriculus. The very fact that, of the 123 infected flies which lived longer than 20 days after the first infective meal, no less than 117 (including the 26 'total' infections) showed infection of the proventriculus lends support to this hypothesis. However, whilst this analysis seems to afford very strong evidence that, in the great majority of flies in which the trypanosome succeeded in establishing itself outside the peritrophic membrane, the infection would have proceeded forward as far as the proventriculus, had the flies lived long enough, there is some evidence that this would not have invariably occurred. In the first place, it will be seen that on two occasions 'gut only' infections were found in flies dissected in the 36th to 40th day period. As proventriculus infections were actually seen by us as early as the 9th and 10th days, and as such large numbers of proventriculus

infections were found in flies dissected before the 25th day, it is difficult to understand why a few flies kept under the same conditions should show 'gut only' infections as late as the 36th–40th day, if the infection had been capable of reaching the proventriculus. In the second place, it seems significant that the only infections found in the attempt to transmit Strain 3 (Table IA) in July, 1936, when the trypanosome appeared to be definitely non-transmissible, were a few 'gut only' infections. In this experiment, of the 435 flies which lived beyond 10 days after the first infecting meal, only 3 were, on dissection, found to contain trypanosomes, and in each case it was a 'gut only' infection.

TABLE VI

Showing the location of trypanosomes in *Glossina palpalis* found to be infected at various intervals after the 1st infecting feed on various transmissible strains of *T. gambiense*. (Compiled from data supplied by van Hoof and Henrard, 1934)

Days after 1st infecting feed	Site of infection		
	Gut only	Gut + proventriculus	Gut + proventriculus + salivary glands
5–10	57	1	Of the 45 flies which on dissection showed invasion of the salivary glands, it was possible by feeding experiments to obtain an approximate date by which the salivary glands had become infected in 12 cases. In 3 of these the flies had become infective on the 17th day, in 1 on the 21st day, in 1 on the 22nd day, in 1 between the 27th and 30th day, in 2 on the 32nd day, and in 4 at some period between the 40th and 80th day
11–15	27	6	
16–20	22	10	
21–25	11	10	
26–30	12	3	
31–35	10	4	
36–40	3		
41–45	5	6	
46–50	4	4	
51–55		1	
56–60			

Before leaving the subject of the significance of the proventriculus, it will be of interest to consider very briefly the considerable mass of data recorded by van Hoof and Henrard (1934) in their second paper on the cyclical transmission of resistant strains of *T. gambiense* by *G. palpalis*. In this work, van Hoof and Henrard publish the results obtained from dissection of the flies employed in 16 successful transmission experiments with various strains of *T. gambiense*. In order to render their observations comparable with ours, we have summarized their data in Table VI, which should be considered together with Table V.

It will be seen that the results obtained by van Hoof and Henrard differ in several important respects from ours, the most notable of which is in the large number of 'gut only' infections as compared with 'gut + proventriculus' infections. If we omit the flies dissected before the 10th day, we find that,

amongst those dissected from the 11th day onwards, 94 exhibited 'gut only' infections, as compared with 44 'gut + proventriculus' infections and 45 'total' infections. In Table VII we have summarized the results of dissecting infected flies which died later than 10 days after their first infecting meal, obtained by ourselves in Liverpool, by Taylor (1932) in Nigeria, and by van Hoof and Henrard (1934) in Leopoldville.

As the Table shows, there is a striking parallelism between the results obtained by us and those obtained by Taylor in each of his two series of experiments conducted at laboratory temperature and incubator temperature (37° C. for 16-24 hours per day during the 4 infecting meals) respectively. In all three sets of experiments, the vast majority (about 90 per cent.) of the infections

TABLE VII

Comparing the results of dissecting infected flies, which had lived more than 10 days after the 1st infected meal, obtained by ourselves with those obtained by Taylor and by van Hoof and Henrard

Author	No. of flies used	No. of infected flies living more than 10 days after 1st infected meal	Site of infection			Percentage of proventricular infections which reached salivary glands
			Gut only	Gut + proventriculus	Gut + proventriculus + salivary glands	
Murgatroyd and Yorke	2,407	152	18 [12 %]	108 [71 %]	26 [17 %]	19.4
Taylor (1932)						
Experiments at lab. temp.	5,954	221	11 [5 %]	155 [70 %]	55 [25 %]	26.2
Incubator experiments	2,246	482	61 [12.7 %]	292 [60.6 %]	129 [26.7 %]	30.6
Van Hoof and Henrard (1934)	1,744	183	94 [51.4 %]	44 [24 %]	45 [24.6 %]	50

which became established in the extraperitrophic space extended forward to reach the proventriculus. The results obtained by van Hoof and Henrard differ strikingly in that in only about half of the cases in which the injection had established itself in the extraperitrophic space was the proventriculus involved. We are unable to offer any explanation for this remarkable discrepancy. Possibly the solution will be found to be in differences of climatic conditions; but obviously the matter merits further investigation.

With the object of obtaining some insight into what happened when our strain of *T. brucei* was becoming non-transmissible, we have summarized in Table VIII the results of dissection of all the flies in our various transmission experiments which lived more than 10 days after the first infecting meal. In this Table we are able to compare the findings in the earlier experiments, when the

strains were readily transmissible, with those of the later experiments, when the strains were not transmissible. We see that in the earlier experiments, when attempts to transmit succeeded, 844 flies lived 11 days or more after the first infecting meal, and were dissected; of these, 112 were found to harbour trypanosomes, 12 in the 'gut only,' 74 in the 'gut + proventriculus,' and 26 in the 'gut + proventriculus + salivary glands.' From this we conclude that, although under the conditions of the experiment only about 13 per cent. of the flies became infected at all, yet in about 90 per cent. of those which did the infection invaded the proventriculus. However, in only about 26 per cent.

TABLE VIII

Comparing the results obtained from dissection of the flies in the experiments in which transmission succeeded with those in which it failed

	Strain	No. of flies alive 11 days after 1st infected meal	No. found to be infected when dissected	Site of infection		
				Gut only	Gut + proventri- culus	Gut + pro- ventriculus + salivary glands
Experiments in which transmission succeeded. Nov. 1932-Mar. 1935	3 [Table IA]	326	50	4	37	9
	5 [Table IIA]	399	32	4	21	7
	6 [Table IIIA]	119	30	4	16	10
		844	112	12	74	26
Experiments in which transmission failed. Jan.-Mar. 1936	3 [Table IA]	321	37	3	34	0
June-Sept. 1936	3 [Table IA]	738	3	3	0	0
	5 [Table IIA]	407	0	0	0	0
		1,145	3	3	0	0

of those in which the trypanosomes reached the proventriculus were the salivary glands invaded.

Glancing at the earliest experiments in which transmission failed, we find that, of 321 flies which were dissected 11 days or more after the first infecting meal, 37 were infected; and in 90 per cent. of these was the proventriculus invaded. In the latest experiments, only 3 of the 1,145 flies which lived 11 days or more after the first infecting meal were infected, and in these the infection was limited to the extraperitrophic space in the gut.

From these observations we conclude that when the strain was transmissible, although only a proportion of the flies became infected, in the great majority of those which did the infection reached the proventriculus. But in only a

proportion of those in which the proventriculus became infected was the cycle of development completed and the salivary glands invaded. In these experiments, therefore, establishment of the trypanosomes outside the peritrophic membrane implied that in all probability the infection would, if the fly survived, travel forward to the proventriculus; but infection of the proventriculus did not by any means imply that the salivary glands would eventually have become invaded had the fly lived long enough.

The first stage in the loss of transmissibility by the trypanosome appeared to be that it lost its capacity to travel forwards to the salivary glands from the proventriculus; the second stage appeared to be that it became unable to extend forwards in the extraperitrophic space as far as the proventriculus; and the final stage that it became unable even to establish itself outside the peritrophic membrane.

Before concluding this paper, reference should be made to recent communications by Roubaud (1935) and Schilling (1936). Roubaud, in a paper dealing with atypical infections of trypanosomes in *Glossina*, adheres to the view which he first put forward in 1909, that in certain cases *T. congolense* can establish itself directly in the salivary tract of *Glossina* without first invading the digestive tract. Schilling, in a letter commenting on Roubaud's communication, raised the question whether trypanosomes belonging to the polymorphic group do not reach the salivary glands directly. After dismissing the hypothesis that the trypanosomes reach the salivary glands by traversing the abdominal cavity from the intestine, Schilling writes: 'N'est-il pas plus vraisemblable, que des trypanosomes très rares entrent immédiatement dans l'hypopharynx, remontent le courant de salive et s'établissent dans le canal central de la glande?' As Roubaud says in reply, this is a plausible theory, but unfortunately it lacks any experimental support.

If Schilling's hypothesis be correct, it is surely a curious coincidence that, almost without exception, flies which exhibit a salivary gland infection likewise exhibit a proventriculus and gut infection. Duke (1933a) records that he found a heavy infection of *T. gambiense* in the salivary glands of a fly, the gut of which was negative. He considers that this must be an exceedingly rare phenomenon, and states that he has never before found the glands infected without finding flagellates in the alimentary canal. So far as we are aware, no other investigator has as yet observed an instance of salivary gland infection without infection of the intestine.

SUMMARY

Evidence is produced that a strain of *T. brucei* isolated from wild *G. morsitans* in 1931, and subsequently maintained in Liverpool by passage through guinea-pigs, partly by blood inoculation and partly by *G. morsitans*, remained transmissible by the tsetse for at least 4 years, and then became non-transmissible.

Analysis of the results of dissecting flies used in the transmission experiments showed that in about 90 per cent. of the cases in which the trypanosomes became established outside the peritrophic membrane the infection spread forwards to the proventriculus.

In only about 20 per cent. of cases in which the proventriculus was involved were the salivary glands also invaded. This could not be explained on the hypothesis that the flies which exhibited only a 'gut + proventriculus' infection had not lived long enough for the trypanosomes to reach the salivary glands. In all the instances in which it was possible to examine the point, the salivary glands had become infected by the 26th day or earlier. This observation agrees with the dictum of Duke that if the salivary glands are going to become invaded they will have done so before the 30th day after the infecting meal. Considerable numbers of flies were encountered in which proventricular infection had persisted long beyond this period without spreading forward to the salivary glands.

In our experiments, therefore, establishment of the trypanosomes in the extraperitrophic space implied that in all probability the infection would reach the proventriculus; but proventricular infection did not necessarily imply that the salivary glands would eventually become infected, as suggested by Taylor (1932).

The stages by which our strain of *T. brucei* became non-transmissible appear to be, firstly, that the trypanosome lost the capacity to extend from the proventriculus to the salivary glands; then, that it lost the capacity to extend forwards in the extraperitrophic space to the proventriculus; and, lastly, that it lost the capacity of establishing itself in the extraperitrophic space.

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STUDIES ON THE HIGHER DIPTERA OF MEDICAL AND VETERINARY IMPORTANCE

A REVISION OF THE SPECIES OF THE GENUS *MUSCA*,
BASED ON A COMPARATIVE STUDY OF THE MALE
TERMINALIA

IV.—A PRACTICAL GUIDE TO THE ORIENTAL SPECIES

(Continued from page 140)

BY

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(Received for publication 3 February, 1937)

Musca pattoni Austen. Ann. & Mag. Nat. Hist., 8th Ser., V, 115, 1910.

SYNONYM : ? *spinosa* Awati.

MALE. HEAD. Vertex very narrow, eyes approximated. MESONOTUM. Four broad black stripes ; R_{4+5} with a row of small bristles on ventral side extending beyond the radio-medial cross-vein. ABDOMEN. Light orange ; terga 1 and 2 light orange, with a narrow, median, dark-brown stripe expanding T-shaped anteriorly ; tergum 3 light orange, with a broader dark-brown median stripe edged with broad yellowish-white patches and marginal white spots ; tergum 4 with median dark stripe much narrower ; tergum 5 mainly silvery, with dark admedian stripes.

MALE TERMINALIA. Fig. 9. ANAL CERCI. Rather long and narrow, with short, rather wide, nipples at inner margin, and a deep emargination between nipple and the rounded outer margin. PHALLOSOME. Short chitinous part extending dorsally and ventrally, with a deep, wide emargination ; membranous part long ; posterior process long, expanded and deeply forked. PARAMERE. Anterior part rounded and deep, with one long and two shorter bristles ; posterior part long and wide and bent at end. FIFTH STERNUM. Wide and long, with a dark area at each side of distal end ; posterior processes short, wide and finely serrated.

FEMALE. HEAD. Vertex wide, almost width of an eye. MESONOTUM. Similar to that of ♂ ; R_{4+5} with a row of bristles as in ♂. ABDOMEN. Orange ; terga 1 and 2 orange, with anterior margin black, and with or without a narrow, dark, median stripe, a dark-brown median patch on posterior border forming an incomplete band ; tergum 3 with a broad, median, dark-brown stripe somewhat T-shaped anteriorly, edged with broad silvery stripes and marginal silvery spots, intervening areas forming narrow, dark, admedian stripes widening out posteriorly to form incomplete posterior band ; tergum 4 similar, but the median dark-brown stripe narrower ; tergum 5 silvery, with admedian dark-brown stripes.

EARLY STAGES. Oviparous, eggs laid singly in patches of cow-dung, the long stalk directed upwards. THIRD LARVA. Anterior spiracles with 6-7 finger-like processes. POSTERIOR SPIRACULAR PLATES. Fig. 8, *c*. Large, densely chitinized and well separated; breathing-slits long, wide and well convoluted.

NOTES. This large haematophagous species is widely distributed in India, Burma and Ceylon, but I have not seen it from any other part of the region; it is common about animals, especially cattle, feeding on secretions, cuts, etc.

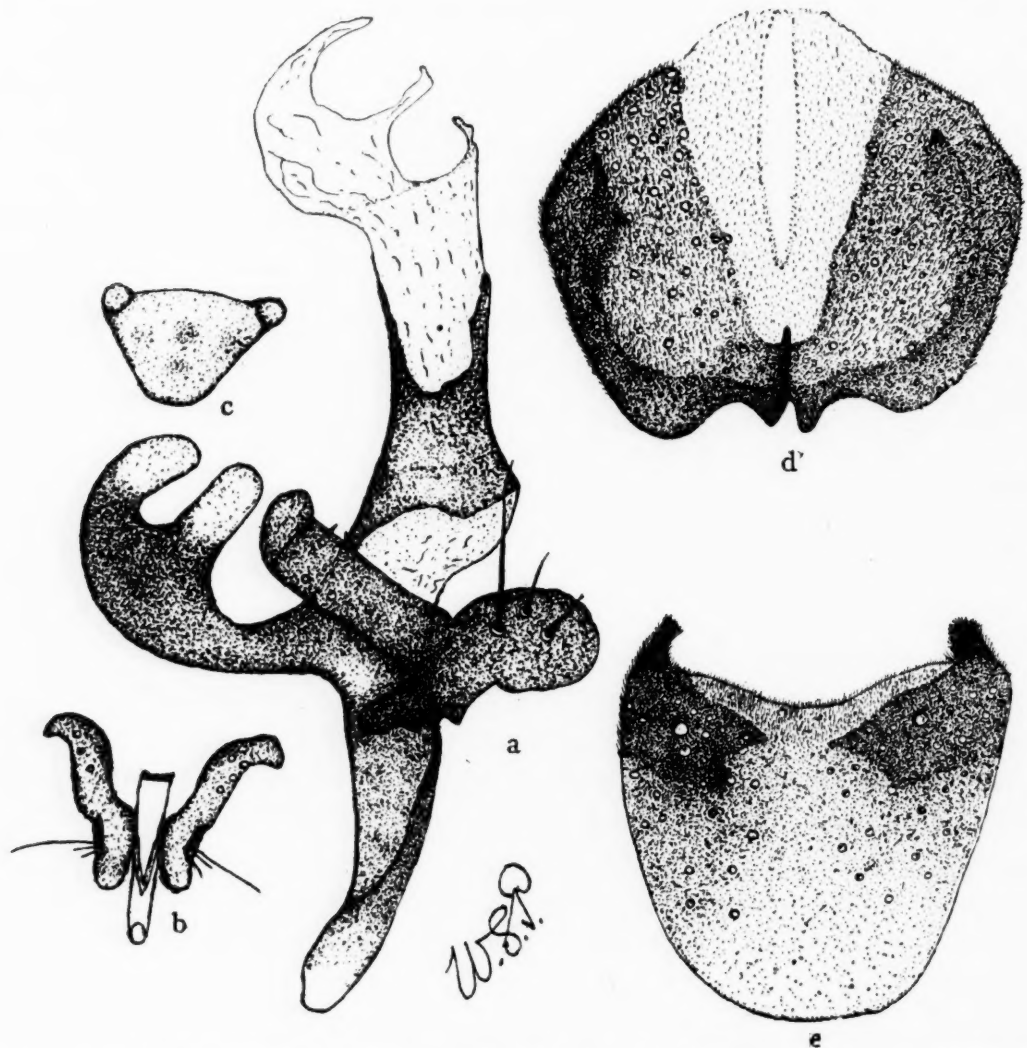


Fig. 9. *a*.—Phallosome and one paramere of *pattoni*; *b*.—Dorsal view of parameres; *c*.—Dorsal view of posterior process of phallosome; *d*.—Anal cerci; *e*.—Fifth sternum.

***Musca spinohumera* Awati. Ind. Jl. Med. Res., V, 167, 1917.**

MALE. HEAD. Vertex narrow, about one-seventh to one-eighth width of an eye. MESONOTUM. Four black stripes; R_{4+5} with a row of small bristles on ventral side extending beyond the radio-medial cross-vein. ABDOMEN. Mostly orange and silvery; terga 1 and 2 almost entirely dark brown, except lateral margins which are light yellow; tergum 3 light orange, with a broad,

median, dark-brown stripe expanding anteriorly in the middle T-shaped ; terga 4 and 5 very similar, but median stripes narrower.

MALE TERMINALIA. Figs. 10 ; 11. ANAL CERCI. Long and wide, outer margin forming a rounded projection, inner margin forming characteristic, long, wide, rounded nipple, with a deep emargination between two margins. PHALLOSOME. Chitinous part long and narrow and with a waist ; membranous part

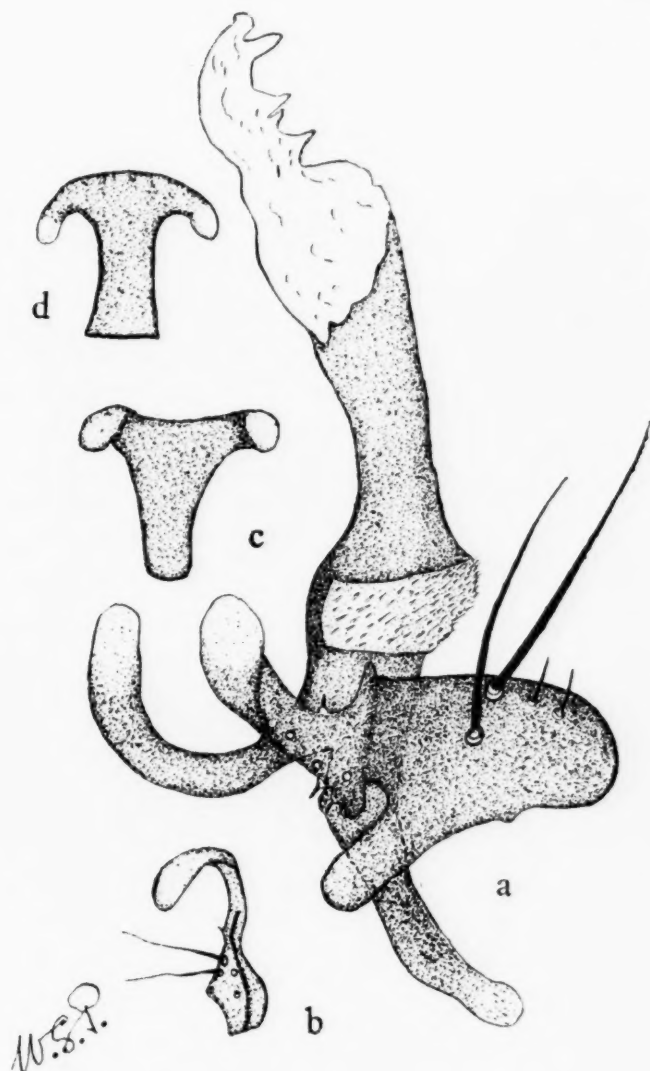


Fig. 10. *a.*—Phallosome and one paramere of *spinohumera* ; *b.*—Dorsal view of right paramere ; *c.*—Dorsal view of posterior process of phallosome ; *d.*—Ventral view of same.

joining two parts with numerous fine spines ; posterior process long, bent and forked. PARAMERE. Anterior part long, wide, and rounded at end, with two long stout bristles and two smaller ones, or only one long and two smaller ; posterior part long, wide and bent at end. FIFTH STERNUM. Wide, with long broad serrated posterior processes.

FEMALE. HEAD. Vertex wide, three-quarters to (almost) width of eye; outer vertical hairs in two rows. MESONOTUM. As in ♂; R_{4+5} with a row of bristles as in ♂. ABDOMEN. Orange and silvery; terga 1 and 2 orange, with a median black stripe and greyish-yellow patches on each side of median line

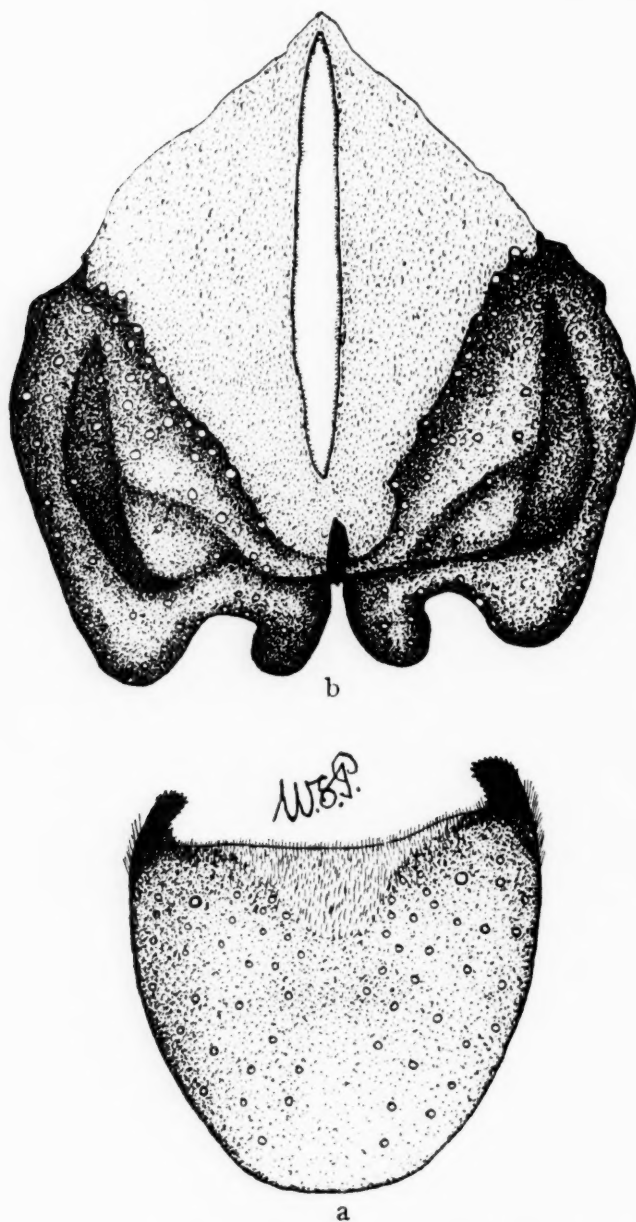


Fig. 11. *a*.—Fifth sternum of *spinothumera*; *b*.—Anal cerci.

on posterior border, anterior border dark brown; tergum 3 with a dark-brown to black median stripe spreading out anteriorly to form an incomplete band, admedian brown triangular stripes and silvery intervening and marginal spots; tergum 4 with a dark median stripe, sometimes incomplete posteriorly, otherwise similar to tergum 3; tergum 5 silvery, with a faint dark median stripe.

EARLY STAGES. Larviparous, second stage larva deposited in cow-dung, one at a time. THIRD LARVA. Anterior spiracles with 5-7 finger-like processes. POSTERIOR SPIRACULAR PLATES. Fig. 2, *d*. Large, densely chitinized and well serrated; breathing-slits long and narrow. Puparium white.

NOTES. This haematophagous species is common along the Indo-Gangetic Plain. It is only found on animals and about cow-dung.

Musca xanthomelas Wiedemann. For the synonymy, notes on the adults, illustrations of the ♂ terminalia and the posterior spiracles of the third larva, see Pt. 2. *Xanthomelas* is widely distributed in the region, extending to Canton and the Island of Hainan. It is oviparous, the egg having a long stalk; the puparium is white.

Musca hervei Villeneuve. For notes on the adults, illustrations of the ♂ terminalia and posterior spiracles of the third larva, see Pt. 2. This haematophagous species is widely distributed in China. I have seen specimens from Shillong, Upper Burma, and from Simla, North India.

Musca illingworthi Patton. Philip. Jl. Sci., XXIII, 323, 1923.

MALE. HEAD. Vertex very narrow, eyes approximated. MESONOTUM. Four broad black stripes; R_{4+5} with a row of bristles on the ventral side extending beyond the radio-medial cross-vein. ABDOMEN. Dark orange and black; terga 1 and 2 dark brown, or black anteriorly and at the sides, and a rather diffuse dark brown patch in the middle line; the antero-lateral and posterior border dark orange; tergum 3 with a broad median black stripe widening anteriorly, a broad silvery stripe on each side, and a large silvery spot at extreme margins, intervening areas dark orange; terga 4 and 5 similar to tergum 3, the admedian brown stripes tending to form a narrow dark posterior band.

MALE TERMINALIA. Fig. 12. ANAL CERCI. Inner margins with rather short, broad, not very prominent nipples; a deep emargination between nipple and outer border, which is broadly rounded. PHALLOSOME. Chitinous part very short and broad; membranous portion long and wide; posterior process long and bent and well forked. PARAMERE. Anterior part long, wide and rounded, with one long stout bristle and one shorter one; posterior part long, wide and bent at the end. FIFTH STERNUM. Long and broad, with dark area at each side of the end; posterior processes short, narrow, pointed and bent inwards.

FEMALE. HEAD. Vertex half width of an eye; a single row of outer vertical hairs. MESONOTUM. Similar to that of ♂; R_{4+5} with a row of bristles as in ♂. ABDOMEN. Terga 1 and 2 similar to that of ♂; tergum 3 with a narrower median dark stripe, the stripes adjacent to it and the marginal spots yellower, the admedian brown stripes broader, more triangular in shape, and extending along the lower border, forming a posterior band interrupted a little.

in the middle ; terga 4 and 5 similar, the admedian brown stripes narrower, and the posterior band on tergum 4 more complete.

EARLY STAGES. Unknown.

NOTES. This species is widely distributed in the Middle East, extending from Malay to the Philippine Islands. It is haematophagous in habit, and is common on and about cattle and cow-dung.

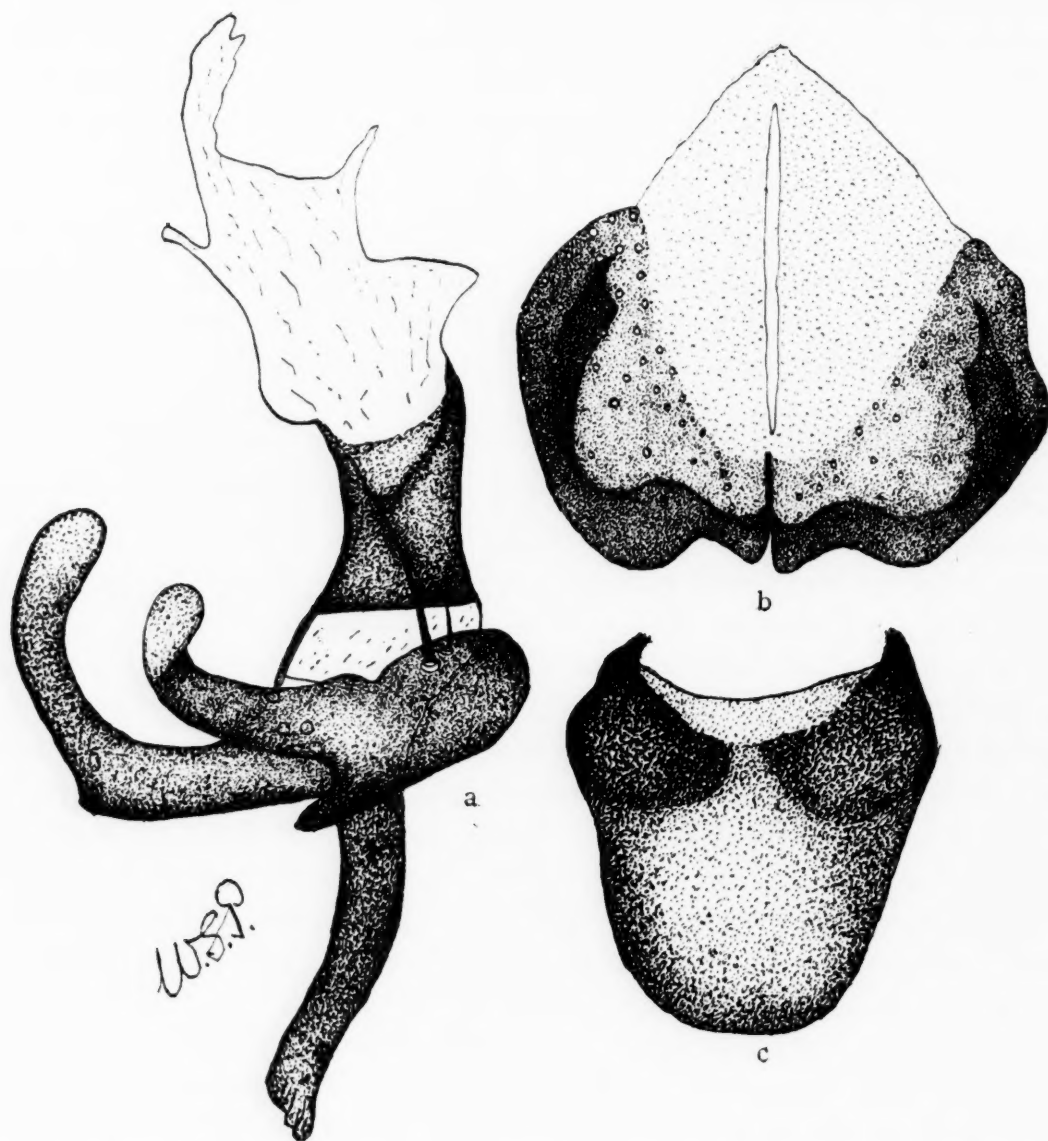


Fig. 12. *a*.—Phallosome and one paramere of *illingworthi* ; *b*.—Anal cerci ; *c*.—Fifth sternum.

Musca fletcheri Patton and Senior-White. Rec. Ind. Mus., XXVI, 574, 1924.

MALE. HEAD. Vertex wide, about one-third width of an eye ; mentum strongly chitinized ; three large and two smaller prestomal teeth (for illustrations of proboscis and prestomal teeth, see Patton, 1933*a*). MESONOTUM. Four broad black stripes, the inner pair ending abruptly some distance before posterior

border of mesonotum ; R_{4+5} with a row of bristles along ventral side, extending beyond the radio-medial cross-vein ; squama without hairs on dorsal surface. ABDOMEN. Terga 1 and 2 mainly black, the posterior border on each side of the median black stripe narrowly yellow ; tergum 3 yellow, with a broad median black stripe narrowly edged by bluish-white stripes, the median stripe spreading out along the middle of the posterior border, forming a narrow band ; tergum 4

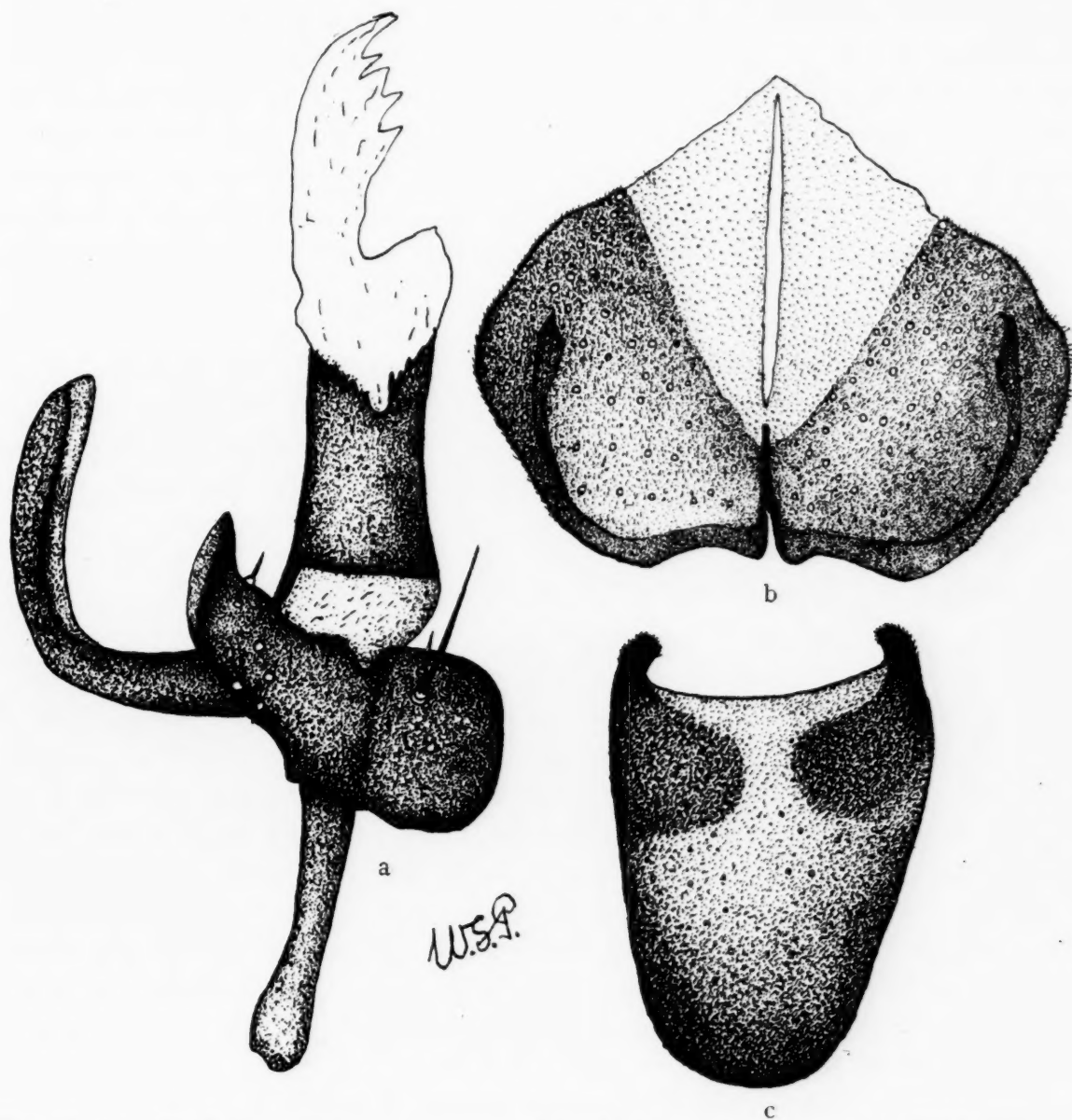


Fig. 13. *a.*—Phallosome and one paramere of *fletcheri* ; *b.*—Anal cerci ; *c.*—Fifth sternum.

similar, but with very narrow median black stripe and dark-orange admedian triangular spots posteriorly ; tergum 5 yellow. First sternum black, remainder orange.

MALE TERMINALIA. Fig. 13. ANAL CERCI. Long and rather wide, inner margin forming small nipples ; emargination between nipple and outer rounded

border shallow. PHALLOSOME. Chitinous part rather short and wide ; membranous part long ; posterior process long and broadly expanded, hood-like, and not forked. PARAMERE. Short, wide and rounded ; posterior part long, broad, bent at the end and with several well-developed spines. FIFTH STERNUM. Long and rather narrow, with black patches at side of distal end ; posterior processes long, broad, bent in and well serrated.

FEMALE. HEAD. Vertex wide, about equal to width of an eye ; outer vertical hairs in two rows ; proboscis as in ♂. MESONOTUM. As in ♂ ; R_{4+5} with bristles as in ♂ ; squama without hairs on dorsal surface. ABDOMEN. Terga 1 and 2 mainly orange, the anterior border black, a broad median black stripe with bluish-white spots on each side ; tergum 3 grey, with a narrower median dark stripe, dark-brown admedian stripes widening out posteriorly ; tergum 4 grey, without any definite median stripe, but a dark patch in rubbed specimens, admedian brown stripes not reaching the anterior border ; tergum 5 grey, with narrow admedian stripes.

EARLY STAGES. Unknown.

NOTES. This species was recorded from Samalkota, eastern coast of India, and also from Shencottah, Western Ghats, and Silchar, Cachar, Assam. Although there is no proof that it is a blood-sucker, the structure of the proboscis strongly suggests that it can draw blood, the prestomal teeth and axial apophysis being well developed.

Musca bakeri Patton. Philip. Jl. Sci., XXIII, 316, 1923.

MALE. HEAD. Vertex very narrow, eyes closely approximated. MESONOTUM. Four broad black stripes ; R_{4+5} with a row of bristles on ventral side extending beyond the radio-medial cross-vein. ABDOMEN. Terga 1 and 2 either entirely black or only the anterior part black, with a broad median black stripe, remainder dark brown ; tergum 3 either light or dark brown, with a broad black median stripe and a narrow black anterior band extending the length of the middle third, a large light silvery-yellow admedian spot or patch, and silvery-yellow patches at the extreme edges ; tergum 4 very similar, except that the marginal patch is larger, and between it and the admedian spot there is a broad, triangular, clove-brown stripe, and in many specimens a narrow black basal band ; tergum 5 exactly similar. Sterna black, forming a characteristic ventral black band.

MALE TERMINALIA. Fig. 14. ANAL CERCI. Long and wide, outer margin rounded and inner forming short nipple-like projection. PHALLOSOME. Chitinous part long and wide ; membrane joining two parts covered with numerous spines ; posterior process long and deeply forked. PARAMERE. Anterior part long and wide, with one long stout bristle and one smaller one ; posterior part long, bent and slightly dilated at end, with several short raised spines. FIFTH STERNUM. Very long and wide, with stout, short, bent, serrated posterior processes.

FEMALE. HEAD. Vertex wide, more than half width of an eye, with two

rows of small outer vertical hairs. MESONOTUM. As in ♂; vein R_{4+5} with bristles as in ♂. ABDOMEN. Greyish, with black bands and stripes. Terga 1 and 2 black, with bluish-grey admedian patches often extending the whole width of the segment and forming a band; tergum 3 grey, with a broad median black stripe and two lateral narrow ones, which broaden out at the lower border

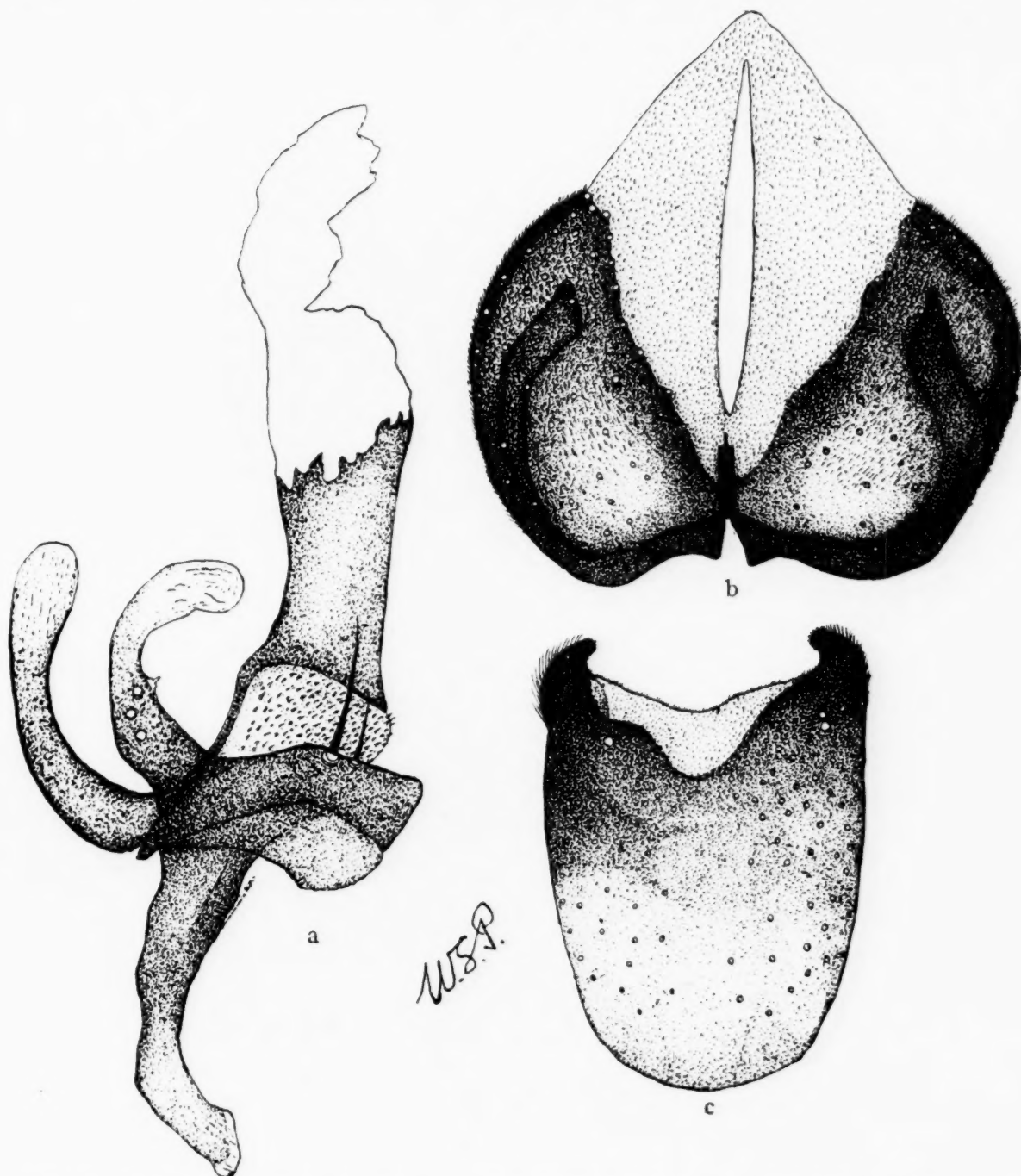


Fig. 14. *a.*—Phallosome and one paramere of *bakeri*; *b.*—Anal cerci; *c.*—Fifth sternum.

forming narrow black bands; there is also a narrow black band along the anterior border; tergum 3 exactly similar, but without an anterior black band; tergum 5 mostly grey.

EARLY STAGES. Unknown.

NOTES. This large haematophagous species is widely distributed in the Philippine Islands ; I have not yet seen it from any other locality.

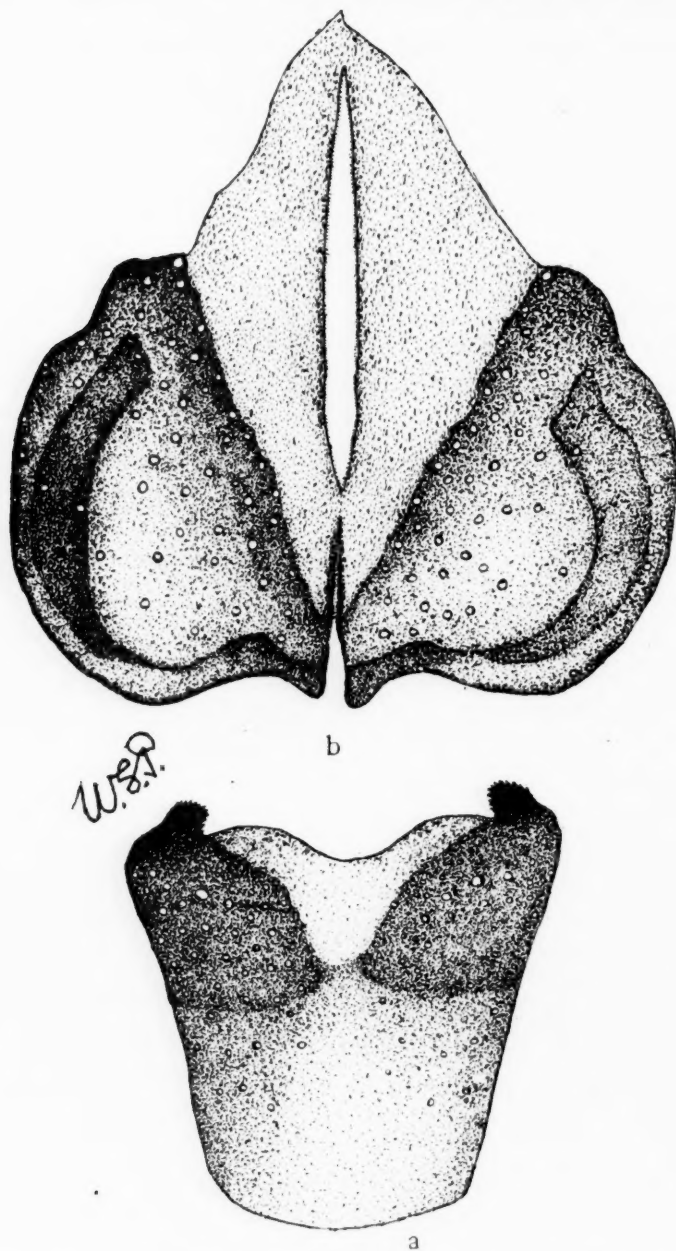


Fig. 15. a.—Fifth sternum of *bezzii* ; b.—Anal cerci.

Musca bezzii Patton and Cragg. Ind. Jl. Med. Res., I, 19, 1913.

SYNONYM : ? *pilosa* Awati.

MALE. HEAD. Vertex very narrow, eyes approximated. MESONOTUM. Four broad black stripes ; R_{4+5} with a row of bristles on ventral side extending beyond the radio-medial cross-vein. ABDOMEN. Orange, with black markings ; terga 1 and 2 black, except the posterior margin on each side of the median stripe, which is orange ; tergum 3 with a broad median black stripe expanding T-shaped

for a short distance along the anterior border, with silvery edging, white marginal spots, the intervening areas orange; tergum 4 with a narrower median black stripe broadly edged with yellowish-white stripes, large marginal yellow spots, and posterior border with a narrow black band; tergum 5 very similar, but without band.

MALE TERMINALIA. Figs. 15; 16. ANAL CERCI. Long and wide, outer margin broadly rounded, inner forming small nipple. PHALLOSOME. Chitinous

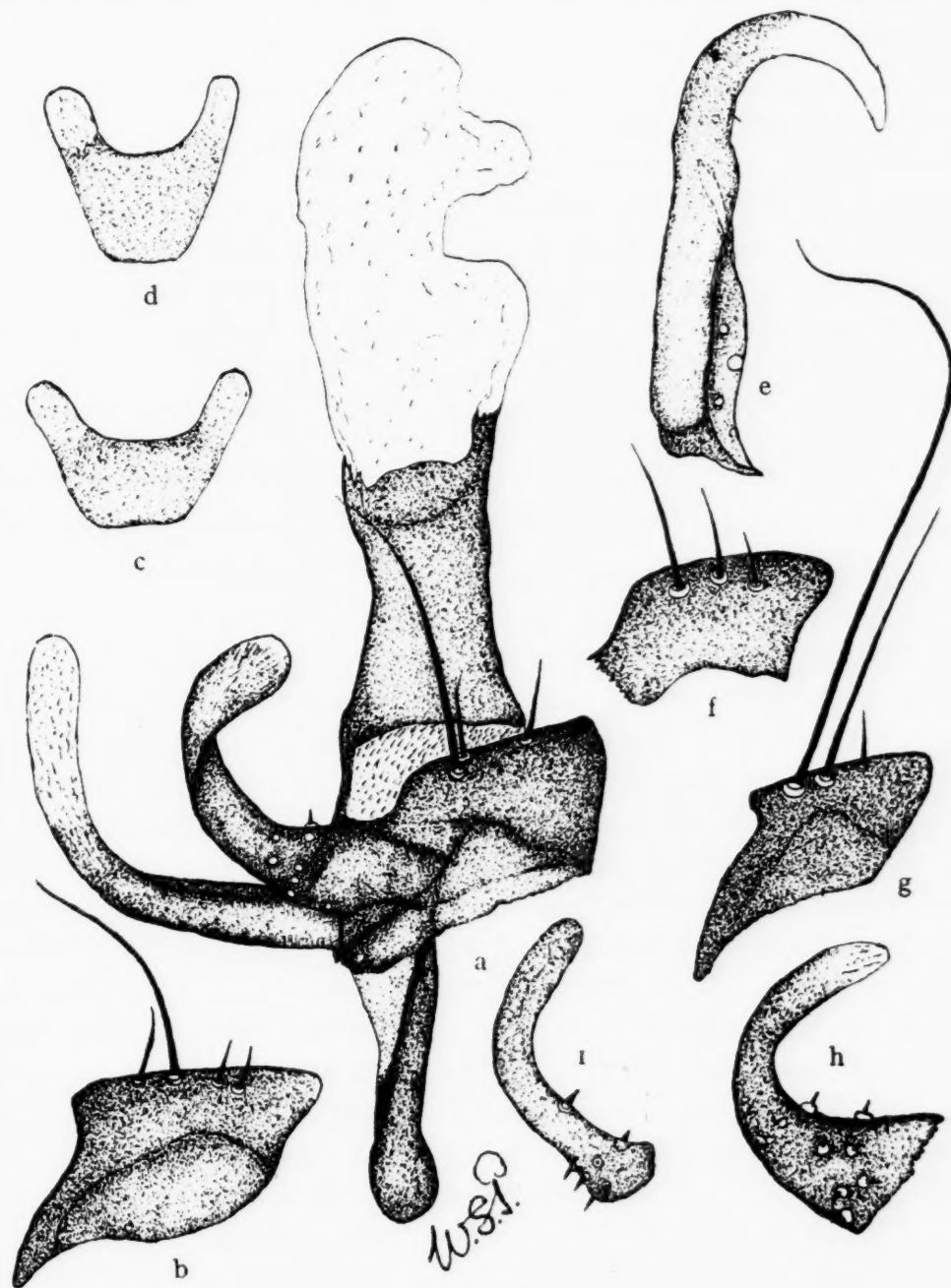


Fig. 16. *a*.—Phallosome and one paramere of *bezzii* (Coonoor, S. India); *b*.—Anterior part of right paramere (Simla, N.-W. India); *c*.—Ventral view of posterior process of phallosome; *d*.—Dorsal view of same; *e*.—Left posterior paramere (Coonoor, S. India); *f*.—Anterior part of right paramere (Darjeeling); *g*.—Anterior part of right paramere (Coonoor, S. India); *h*.—Posterior part of paramere (Darjeeling); *i*.—Posterior part of paramere (Coonoor, S. India).

part of medium length ; membrane between two parts with numerous fine spines ; posterior process long, bent and forked. PARAMERE. Anterior part short and wide, with either one or two long bristles (one sometimes particularly long), and 1-3 smaller ones ; posterior part long and bent. FIFTH STERNUM. Long and wide, dark at the distal sides ; posterior processes short, very wide and serrated.

FEMALE. HEAD. Vertex wide, about half width of an eye ; outer vertical hairs in 4-6 rows. MESONOTUM. Similar to that of ♂ ; R_{4+5} also with hairs as in ♂. ABDOMEN. Grey, with black stripes and bands ; terga 1 and 2 either entirely black or with admedian bluish-grey spots near posterior border ; tergum 3 with a broad black median stripe broadly edged with bluish-grey spots, triangular admedian black stripes, broadening out posteriorly, and marginal silvery patches ; tergum 4 very similar, but stripes narrower ; tergum 5 similar.

EARLY STAGES. Larviparous, deposits a second stage larva one at a time in cow-dung. THIRD LARVA. Anterior spiracles with 8-9 finger-like processes. POSTERIOR SPIRACULAR PLATES. Large, densely chitinized ; breathing-slits long, very narrow and sinuous (fig. 8, a).

NOTES. This species is widely distributed in India at altitudes of 1,500 feet and upwards.

Musca greeni Patton. Ann. Trop. Med. and Parasitol., XXVII, 477, 1933.

MALE. HEAD. Vertex linear ; eyes with minute scattered hairs. MESONOTUM. Four black stripes. R_{4+5} with a row of bristles along ventral surface, extending beyond the radio-medial cross-vein. ABDOMEN. Terga 1 and 2 orange, with a median narrow black stripe forming a black patch anteriorly ; tergum 3 orange, with a narrow median black stripe expanding T-shape anteriorly, a silvery patch on each side and margins silvery ; tergum 4 dark orange, with a dark median stripe, a broad greyish-yellow stripe on each side, a greyish patch at each margin, and an admedian dark orange stripe ; tergum 5 very similar.

MALE TERMINALIA. Illustrated in another paper (1933c). ANAL CERCI. Free border only slightly emarginated, outer ends broadly rounded, inner produced into rather short broad nipples. PHALLOSOME. Chitinous part long ; the membrane joining the two parts with numerous spines ; posterior process long, broadly expanded and deeply forked. PARAMERE. Anterior part long and broadly rounded, with two small and one long bristle ; posterior portion long, broad and forked. FIFTH STERNUM. Long and broad, about posterior half dark ; posterior processes short, broad and bent inwards.

FEMALE. HEAD. Vertex wide, about three-quarters width of an eye ; outer vertical hairs in two rows ; eyes with very few minute scattered hairs only visible under high power. MESONOTUM. As in ♂ ; R_{4+5} with bristles as in ♂. ABDOMEN. Orange, with extensive greyish-white markings ; terga 1 and 2 orange, with a narrow median black stripe expanding anteriorly to form a large dark band, white patches at side often forming greyish-white bands on

each side of median stripe ; tergum 3 dark orange, dark median stripe, greyish-white stripe on each side, a large dark-brown triangular patch with base at posterior border of tergum, and white patches at sides ; tergum 4 very similar, but median stripe a broad, triangular, dark-brown patch ; tergum 5 very similar.

EARLY STAGES. Unknown.

NOTES. As far as I know at present, *greeni* is found only in Malay, but there is every likelihood that it may be more widely distributed further east. It is haematophagous in habit and is common on and about animals, especially cattle in the field. It can be distinguished from *bezzii* by noting that the ♂ has scattered microscopic hairs on the eyes, whereas the eyes of the ♂ *bezzii* are bare ; terga 1 and 2 are almost entirely light orange, whereas in *bezzii* they are almost entirely black. The eyes of the ♀ *greeni* are also minutely haired, while those of the ♀ *bezzii* are bare ; terga 1 and 2 are also mainly orange, while the same terga of the ♀ *bezzii* are black.

Musca autumnalis De Geer. For the synonymy, notes on the adults, illustrations of the ♂ terminalia and the posterior spiracles of the third larva, see Pt. 2. This species is found only in north-west India and in Kashmir.

Musca inferior Stein.

SYNONYM : *Philaematomyia gurneyi* Patton and Cragg.

MALE. HEAD. Vertex wide, about one-fifth width of an eye ; mentum enlarged and strongly chitinized ; four strong and two smaller prestomal teeth (see Patton, 1933a). MESONOTUM. Four broad black stripes ; vein R_{4+5} has a row of small bristles along the ventral surface extending beyond the radio-medial cross-vein. (Squama with many long delicate hairs on upper surface.) ABDOMEN. Terga 1 and 2 black or dark brown, with posterior admedian bluish-grey spots ; tergum 3 with a broad black median stripe, edged with broad slate-blue stripes, narrow clove-brown admedian stripes and marginal silvery spots ; tergum 4 very similar, but with a narrower black median stripe, and the admedian brown stripes extending on to the posterior border of tergum ; tergum 5 very similar.

MALE TERMINALIA. Fig. 17. Phallosome illustrated in Pt. 1. ANAL CERCI. Long and wide ; long hairy nipple on inner border, outer border rounded. PHALLOSOME. Chitinous part short ; posterior process long, bent, expanded hood-like and forked. PARAMERE. Anterior part long, usually with two long and one shorter bristle ; posterior part long and bent upwards and slightly expanded. FIFTH STERNUM. Long and broad, the posterior processes wide, serrated and bent inwards.

FEMALE. HEAD. Vertex wide, about half width of eye ; proboscis as in ♂. MESONOTUM. As in ♂ ; R_{4+5} also with hairs as in ♂. Squamae with hairs as in ♂. ABDOMEN. Terga 1 and 2 black, with large slate-blue spot on posterior border on each side of middle line ; tergum 3 with a rather narrow

black median stripe, broadly edged with slate-blue patches, dark admedian stripes and silvery margins; tergum 4 similar, but the median stripe narrower and the admedian dark stripes produced along the posterior border; tergum 5 very similar to tergum 4.

EARLY STAGES. Unknown. One specimen was bred by Senior-White from a patch of cow-dung.

NOTES. *Musca inferior* is readily identified by the characters of its proboscis, which is structurally adapted for scratching and tearing through skin and for

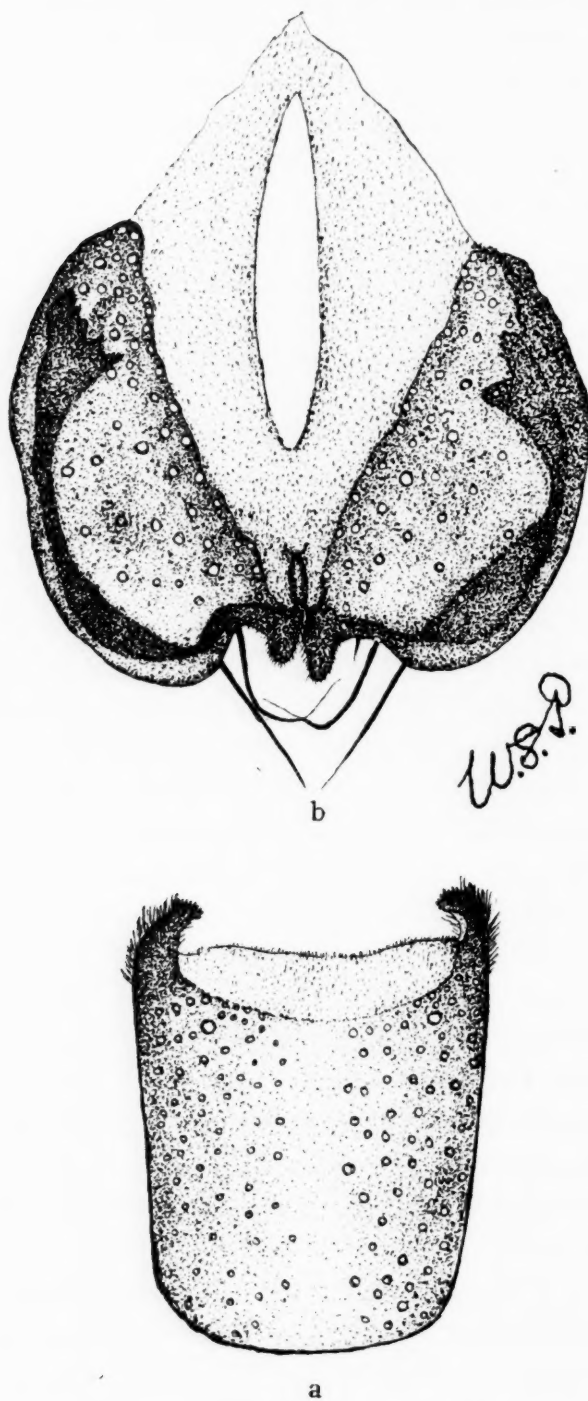


Fig. 17. a.—Fifth sternum of *inferior*; b.—Anal cerci.

drawing blood, and by the presence of hairs on the squama, being the only species which has such hairs. It is widely distributed in the region; I have seen many specimens from the Island of Hainan, collected by Mr. Ch'i Ho.

Musca dasyops Stein. In the paper on the Ethiopian species (1936) I provisionally placed *dasyops* in the *lusoria* group, pending the examination of the ♂ terminalia. Since then, through the generosity of Dr. Villeneuve, who gave me one of the two specimens of the ♂ sex which he possesses, I have studied the ♂ terminalia, and I find that *dasyops* belongs to the *sorbens* group. I am giving here a short description of the ♂ terminalia, with illustrations, so that

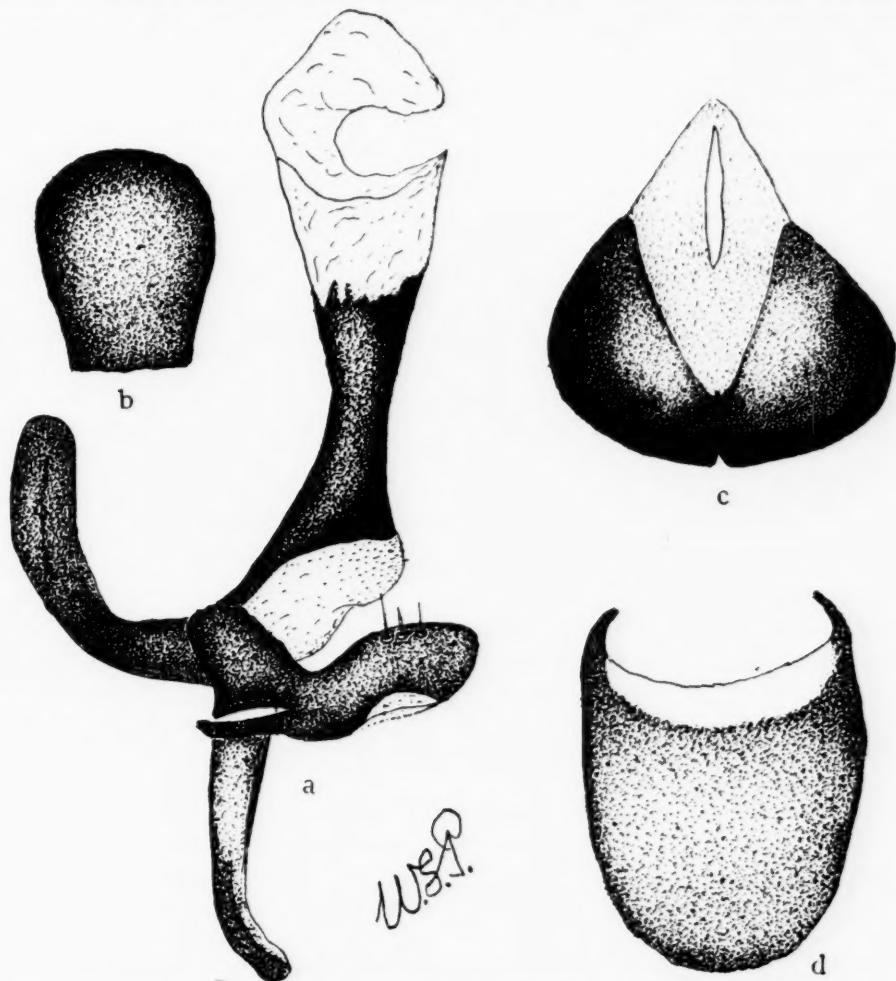


Fig. 18. *a.*—Phallosome and one paramere of *dasyops*; *b.*—Dorsal view of posterior process; *c.*—Anal cerci; *d.*—Fifth sternum.

others may be able to identify it. In an earlier paper (1923*b*) I recorded *dasyops* from south-west China, but I now know that I confused it with a very similar allied species which also belongs to the *sorbens* group. I have recently had the opportunity of examining fresh specimens of the Chinese species collected by Mr. Ch'i Ho, from Hwashan, Shensi. This new Chinese species will in due course be described by Mr. Ch'i Ho.

MALE TERMINALIA. Fig. 18. **ANAL CERCI.** Very similar to those of the other species of this group, the outer margins markedly rounded.

PHALLOSOME. Chitinous part long and narrow, with a marked waist ; membrane joining two parts with many fine spines ; posterior process long and markedly expanded. PARAMERE. Anterior part long and narrow, with three short bristles ; posterior part raised to form a rounded blunt cone, similar to that of *albina*. FIFTH STERNUM. Long and broad, the posterior processes long, narrow and bent inwards.

KEY TO THE ♂♂ OF THE ORIENTAL SPECIES BASED ON EXTERNAL CHARACTERS

1. Vertex wide, from one-third to one-half width of eye 2
Vertex approximately one-third or much less width of eye 3
2. Proboscis with mentum much enlarged, strongly chitinized and bulbous.
Vein R_{4+5} with small bristles on lower surface extending beyond radio-medial cross-vein *fletcheri*
Proboscis with mentum normal. Vein R_{4+5} without small bristles extending beyond the radio-medial cross-vein *domestica*
3. Squama with hairs on the upper surface *inferior*
Squama without hairs on the upper surface 4
4. Mesonotum metallic and without stripes 5
Mesonotum non-metallic and with stripes... .. 7
5. Eyes densely hairy *vitripennis*
Eyes bare 6
6. Sternopleural bristles wanting *albina*
Sternopleural bristles present *tempestiva*
7. Eyes with few microscopic hairs 8
Eyes bare 9
8. Sterna yellow. At present known only from India *gibsoni*
Sterna and adjacent parts of terga black *hervei*
9. Mesonotum with two broad black stripes 10
Mesonotum with four black stripes 14
10. Sterna all black. At present known only from Ceylon *lucens*
Sterna yellow 11
11. Vertex very narrow, eyes closely approximated 12
Vertex wider, eyes well separated 13
12. Small grey species, with dark bands and stripes on abdomen. R_{4+5} without hairs on ventral side extending beyond radio-medial cross-vein *fasciata*
A medium-sized species. R_{4+5} with hairs on ventral side extending beyond radio-medial cross-vein. At present known only from South India *villeneuvei*
13. Thorax grey ; terga 1 and 2 of abdomen sometimes dark orange, with a dark median stripe *sorbens*
Thorax bluish-grey ; terga 1 and 3 of abdomen dark orange to black *vetustissima*
14. Abdomen entirely orange *ventrosa*
Abdomen variously coloured, either orange, grey or olive-green, with dark stripes and bands 15
15. Vein R_{4+5} without small bristles on ventral side extending beyond radio-medial cross-vein 16
Vein R_{4+5} with small bristles on ventral side extending beyond radio-medial cross-vein 23
16. Vertex approximately one-third eye-width... .. *vicina*
Vertex much less than one-third eye-width 17
17. Palps light orange ; mentum of proboscis bulbous ; abdomen olive-green *crassirostris*
Palps black ; mentum normal ; abdomen mainly orange 18

18. Large faceted area of eye well marked ; vertex linear ; abdomen orange, with silvery markings *xanthomelas*
 No marked large faceted area on eyes ; abdomen orange, with dark stripes and silvery patches 19
19. A strong bristle about middle of posterior aspect of fore tibia ; mentum of proboscis rather strongly chitinized. A small species, with rather a silvery abdomen *conducens*
 Species without a strong bristle at middle of posterior aspect of fore tibia ; mentum normal 20
20. Median mesonotal stripes anterior to suture narrow ; terga 1 and 2 of abdomen black. A small species *craggi*
 Median mesonotal stripes anterior to suture not narrow. Larger species 21
21. Vertex about one-seventh to one-eighth width of an eye. Terga 1 and 2 of abdomen light to dark orange, with a broad median black stripe... .. *nebulo*
 Vertex linear, eyes closely approximated 22
22. Terga 1 and 2 of abdomen dark brown to black ; tergum 3 with silvery patches at sides. Only found in Kashmir and adjacent parts *autumnalis*
 Terga 1 and 2 light orange, with a dark median area at posterior border ; tergum 3 always without silvery patches at sides *yerburyi*
23. All sterna black. At present known only from the Philippine Islands *bakeri*
 All sterna mainly orange 24
24. Abdomen pollinose yellow with few dark markings ; mentum of proboscis well chitinized *planiceps*
 Abdomen orange, with extensive dark markings consisting of stripes and bands, or grey with dark markings ; mentum normal... .. 25
25. Eyes well separated ; vertex about one-seventh width of an eye. At present known only from India *spinohumera*
 Eyes closely approximated ; vertex linear 26
26. A medium-sized bluish-grey species ; thorax slate-blue ; abdomen mainly grey, with black markings ; mentum of proboscis slightly thickened *senior-whitei*
 No such species 27
27. Eyes minutely and sparsely haired, hairs seen only with a high power *greeni*
 Eyes bare 28
28. Terga 1 and 2 of abdomen black. At present known only from India *bezzii*
 Terga 1 and 2 either light or dark orange 29
29. Terga 1 and 2 dark brown or black anteriorly and at sides, and a dark-brown patch in middle *illingworthi*
 Terga 1 and 2 light orange, with a narrow median dark-brown stripe. At present known only from India *pattoni*

KEY TO THE ♀♀ OF THE ORIENTAL SPECIES BASED ON EXTERNAL
 CHARACTERS

1. Mesonotum and abdomen metallic green, the former with four black stripes ; terga 1 and 2 of abdomen black ; eyes densely hairy *vitripennis*
 Thorax without metallic sheen, and with either two or four dark thoracic stripes 2
2. Sternopleural bristles wanting *albina*
 Sternopleural bristles present 3
3. Mesonotum with two black stripes 4
 Mesonotum with four black stripes 8
4. Stripes broad and always divided Y-shape anterior to mesonotal suture 5
 Stripes very broad and not so divided 6
5. Thorax grey. Abdomen grey, with dark brown and yellow stripes ; terga 1 and 2 either entirely dark orange or median area only dark *sorbens*
 Thorax blue. Abdomen bluish, with black stripes and bands ; terga 1 and 2 black *vetustissima*

6.	Sterna black. A small species, at present known only from Ceylon	... lucens
	Sterna orange	7
7.	Vein R ₄₊₅ with small bristles on ventral side extending beyond radio-medial cross-vein	... villeneuvi
	Vein R ₄₊₅ without small bristles on ventral side extending beyond radio-medial cross-vein	... fasciata
8.	Squama with hairs on upper surface	... inferior
	Squama without such hairs	9
9.	Mentum of proboscis either bulbous or strongly chitinated	10
	Mentum not bulbous or strongly chitinated	14
10.	Palps yellowish ; mentum bulbous. Abdomen olive green	... crassirostris
	Palps dark grey	11
11.	Presutural dorsocentral bristles small. Vertex wide, about equal to width of an eye. A large species	... fletcheri
	Presutural dorsocentral bristles well developed. Smaller species	12
12.	Vein R ₄₊₅ with small bristles on ventral side extending beyond radio-medial cross-vein	... 13
	Vein R ₄₊₅ without such bristles. Fore tibia with a well-developed bristle about middle of posterior aspect. A small species	... conducens
13.	Vertex wide, almost half width of an eye. A yellow and silvery species	planiceps
	Vertex wide, about equal to width of eye. A bluish-grey species with black markings	... senior-whitei
14.	Sterna black	15
	Sterna mainly light orange	16
15.	Sterna black, the adjacent edges of terga not black. At present known only from the Philippine Islands...	... bakeri
	Sterna and adjacent edges of terga black	hervei
16.	Vein R ₄₊₅ without small bristles on ventral side extending beyond radio-medial cross vein	... 17
	Vein R ₄₊₅ with small bristles on ventral side extending beyond radio-medial cross-vein	25
17.	Median pair of mesonotal stripes anterior to suture narrow ; abdomen dark orange with dark stripes	... craggi
	Median pair of mesonotal stripes not narrower anterior to suture	18
18.	Abdomen entirely orange	... ventrosa
	Abdomen either orange or grey with dark stripes and bands	19
19.	Terga 1 and 2 black	20
	Terga 1 and 2 orange, with dark median stripe	21
20.	Terga 1 and 2 entirely black. A small species, at present found only in Kashmir and the North-West Frontier of India	... tempestiva
	Terga 1 and 2 black, with two bluish-grey spots on posterior border on each side of middle line. A large species, at present found only in Kashmir	... autumnalis
21.	Outer vertical hairs in one row	22
	Outer vertical hairs in two rows. Abdomen orange, with silvery spots and dark posterior bands	... xanthomelas
22.	Vertex equal to about three-quarters width of eye	23
	Vertex about equal to width of eye...	24
23.	Thorax bluish. All terga of abdomen mainly bright orange ; terga 2 and 3 with a dark median stripe and well-marked silvery stripes on each side at margins	... yerburyi
	Thorax greyish. All terga not so conspicuously orange but much more silvery ; tergum 2 with extensive silvery markings, a broad median stripe, dark admedian stripes and silvery margins	... nebulo
24.	Terga 1 and 2 dark orange, remaining terga greyish, with dark markings and some blue sheen	... domestica
	Abdominal terga more extensively orange, especially 1, 2 and 3	vicina

25. Eyes with scattered microscopic hairs. At present known only from
 India *gibsoni*
 Eyes bare 26
26. Terga 1 and 2 of abdomen either dark orange or black 27
 Terga 1 and 2 light orange, with a median dark stripe 28
27. Terga 1 and 2 black, with bluish-grey admedian patches. At present
 known only from India *bezzii*
 Terga 1 and 2 dark brown or black anteriorly and at sides, and brown in
 middle *illingworthi*
28. Radius at base with less than four hairs on upper surface. At present
 known only from India *pattoni*
 Radius at base with four or more such hairs 29
29. Radius at base with four hairs, one usually long. At present known only
 from Assam and Malaya *greeni*
 Radius at base with 5-7 hairs. Larviparous, puparium white. At
 present known only from India *spinohumera*

I had hoped in this paper to give a separate key to the Malayan species, but owing to lack of space this is not now possible. In the final paper in this series I shall deal with the Australian species.

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(To be concluded)

A DESCRIPTION OF A NEW SPECIES OF AMPHISTOME, *CHIORCHIS PURVISI*, WITH NOTES ON THE CLASSIFICATION OF THE GENERA WITHIN THE GROUP

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(Received for publication 1 February, 1937)

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Thirty-five specimens, preserved in formalin, of the amphistome described below were obtained from a tortoise (*Heosemys grandis*) from Kedah, Malaya, and were presented to the Liverpool School of Tropical Medicine by G. B. Purvis, Esq., F.R.C.V.S.

DESCRIPTION OF PARASITE

External characters. Most of the specimens appear to have kept their shape. The outline of the body varies according to the state of maturity. In immature specimens (fig. 2) the form is rather fusiform; in mature (figs. 1, 3), it is a long oval, tapering towards the rounded anterior extremity. The ends are bent somewhat ventrally, so that the ventral surface is flat or concave, and the dorsal surface convex both longitudinally and transversely (fig. 3). The length varies from 3 mm. to 5 mm.; the breadth from 500 μ to 3 mm., the greatest diameter being near the middle. The maximum thickness is from 750 μ to 2 mm. The worms are white in colour. The oral sucker is terminal, and there are papillae surrounding the mouth. The rest of the cuticle is smooth.

The acetabulum is subterminal and relatively large. It is circular in shape, and in adult specimens has a diameter of about 1.3 mm. Its smaller circular aperture points ventro-posteriorly, and averages about 500 μ in diameter. The depth of the ventral sucker is about 1 mm.

Alimentary system. The mouth is surrounded by small papillae, and leads into the oral sucker. This is a muscular organ, which is flattened ventro-dorsally and also dilated posteriorly, where the oesophagus and oral diverticula arise. The average depth of the oral sucker is 450μ . The diverticula are paired, and each one arises independently from the posterior and dorso-lateral aspect of the oral sucker. They are irregularly globular in shape, and are as large as the oral sucker. In sections, the diverticula and the surrounding tissue have shrunk away from each other, leaving a large clear space between them.

The oesophagus (fig. 4) arises from the posterior and ventro-median aspect of the sucker, and has a length of from 800μ to 1 mm. It pursues a rather sinuous course. The anterior third is C-shaped, with the convexity towards the ventral surface of the worm; at first it runs ventrally and posteriorly, and then curves dorsally and posteriorly. The middle third is very tortuous. It first runs dorsally, and then bends abruptly to run ventrally. The anterior and middle thirds of the oesophagus are thin-walled and have an inner circular and an outer longitudinal muscular layer. The diameter is from 80μ to 130μ .

The posterior third is dilated to form the muscular pharynx. It is always directed dorsally and posteriorly. A cross-section through the pharynx (fig. 5) shows about nine well-marked concentric muscle bundles, and an outer longitudinal muscular layer. The diameter is from 230μ to 270μ . The intestinal caeca arise almost at right angles from the pharynx, and then curve gently to run dorsally. They pass dorsal and median to the vitellaria, and dorsal and lateral to the testes, diverging slightly before they terminate just in front of the acetabulum. In no specimen did the caeca reach the level of the acetabulum.

Male genitalia. The testes are oval or round, slightly lobed, and measure about 500μ , each being enclosed in a definite capsule. They are intra-caecal, and diagonally placed. The anterior testis is in the equatorial region, slightly overlapping one caecum, and the posterior testis is just anterior to the acetabulum, slightly overlapping the other caecum. From each testis rises a vas efferens, and these unite in front of the anterior testis to form the vas deferens. This is a greatly convoluted tube, well supplied with muscular tissue. Its coils are situated dorsal and posterior to the cirrus pouch, and it terminates by entering the dorsal part of the cirrus sac. The latter contains the seminal vesicle, the pars prostatica and the cirrus. The duct leading into the genital atrium is very short, and opens at the base of a well-marked genital papilla. The genital atrium is surrounded by a thick muscular bulb. The genital pore is oval in shape, and in all specimens is posterior to the bifurcation of the caeca.

Female genitalia. The ovary is round and smooth, and in mature specimens measures about 300μ . It lies in the mid-line just median to the posterior testis, and may be either in front of, or on a level with, the anterior border of the posterior testis. The oviduct takes origin from the dorsal aspect of the ovary, and turns dorsally to penetrate the shell-gland. The latter is somewhat smaller

than the ovary, and lies dorsal and slightly posterior to it. The oviduct, together with the vitelline duct, forms an oötype in the substance of the shell-gland. From this, the uterus emerges, and immediately gives off Laurer's canal. The latter passes dorsal and slightly anterior, to open just lateral to the mid-line. The uterus is situated intra-caecally, and runs anteriorly. In immature specimens it pursues a very convoluted course. In gravid worms, the intra-caecal space is full of eggs, practically obscuring the testes, ovary and shell-gland. The uterus opens into the genital atrium at the base of the genital papilla. It contains numerous eggs; the uterine eggs are oval, operculated, and measure from 100μ to 130μ in length by 40μ to 50μ in breadth.

The vitelline glands consist of lateral clusters of irregularly rounded acini. In all specimens they were extra-caecal, and extend from behind the level of the genital pore to just anterior to the acetabulum, ending at the same level as the gut caeca.

The excretory system. This could not be satisfactorily demonstrated owing to difficulties in staining. Two main trunks are visible, viz., one on each side in the extra-caecal area. They run practically the whole length of the worm, from the anterior extremity to the upper border of the acetabulum. At the level of the lower border of the oral diverticula, each main trunk is joined, almost at right angles, by a smaller vessel which appears to anastomose with its fellow of the opposite side in the region of the oral sucker. The excretory bladder is situated dorsal to the acetabulum. The short excretory duct rises from the posterior part of the bladder, runs slightly posteriorly, and opens on the dorsal surface of the worm, on a level with the middle of the acetabulum.

DIAGNOSIS

As these parasites possess paired oral diverticula, they are clearly referable to the subfamily *Cladorchinae*. The presence of a pharynx, and the absence of a genital sucker and of papillae on the body and acetabulum, place the worm within the genus *Chiorchis* Fischöeder, 1901, in our own classification. The species differs from all others hitherto recorded, but is most closely related to the *Pseudodiscus hawkesii* = *Chiorchis hawkesii* (Cobbold, 1875).

It differs from *P. hawkesii* in the following points:—

1. The absence of a cirrus pouch in *P. hawkesii*.
2. The gut caeca in *P. hawkesii* apparently always extend to the equator of the acetabulum, and even posterior to it. In *C. purvisi* sp. nov. they never extend to the acetabulum. Furthermore, Stiles and Goldberger (1910) note that in *P. hawkesii*, at the level of the equator, the caeca approach close to the corresponding dorso-lateral aspect of the anterior testis. This has not been observed in *C. purvisi* sp. nov. In *P. hawkesii* the testes are tandem or diagonal, situated in the equatorial region and with over-lapping zones. Lobulation is well marked. In *C. purvisi* sp. nov. they are always diagonally

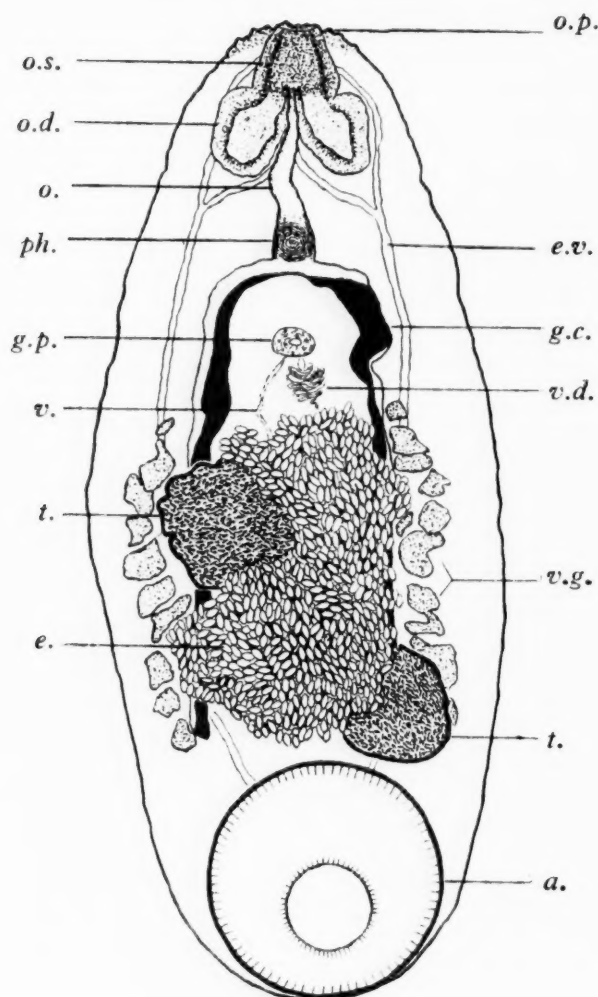


FIG. 1

FIG. 1. *Chiorchis purvisi* sp. nov. Mature specimen, ventral view. (\times about 18.)

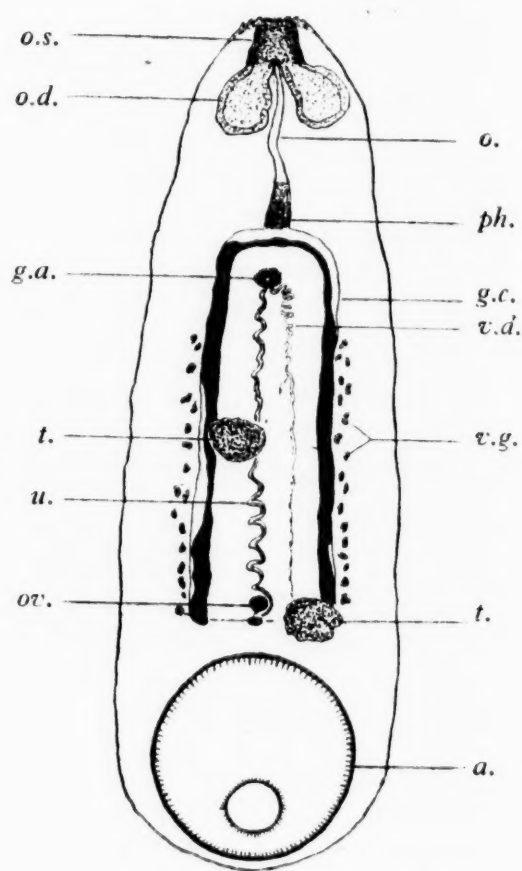


FIG. 2

FIG. 2. *Chiorchis purvisi* sp. nov. Immature specimen, ventral view. (\times about 28.)

EXPLANATION OF LETTERING

a., acetabulum.	g.p., genital pore.	s.g., shell gland.
c.p., cirrus pouch.	L.c., Laurer's canal.	t., testes.
e., eggs.	o., oesophagus.	u., uterus.
e.b., excretory bladder.	o.d., oral diverticulum.	v., vagina.
e.p., excretory pore.	o.p., oral papillae.	v.d., vas deferens.
e.v., excretory vessel.	o.s., oral sucker.	v.g., vitelline glands.
g.a., genital atrium.	ov., ovary.	
g.c., gut caeca.	ph., pharynx.	

placed, one in the equatorial region, and the other in the posterior part of the worm. The zones are never in contact, and lobulation is not marked.

It also bears a somewhat close resemblance to *Cleptodiscus reticulatus* Linton, 1910, but differs from it in that (a) *Cleptodiscus reticulatus* has no muscular pharynx, and (b) the testes are in tandem and equatorial.

We take pleasure in naming our species in honour of G. B. Purvis, Esq., F.R.C.V.S., late Veterinary Surgeon to the Government of the Federated Malay States, who collected the material and presented it, as part of a large collection of helminths, to the Liverpool School of Tropical Medicine.

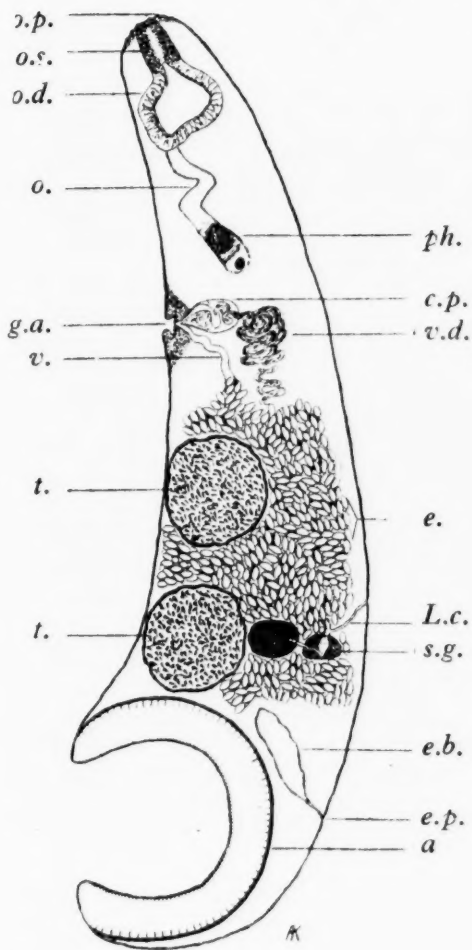


FIG. 3

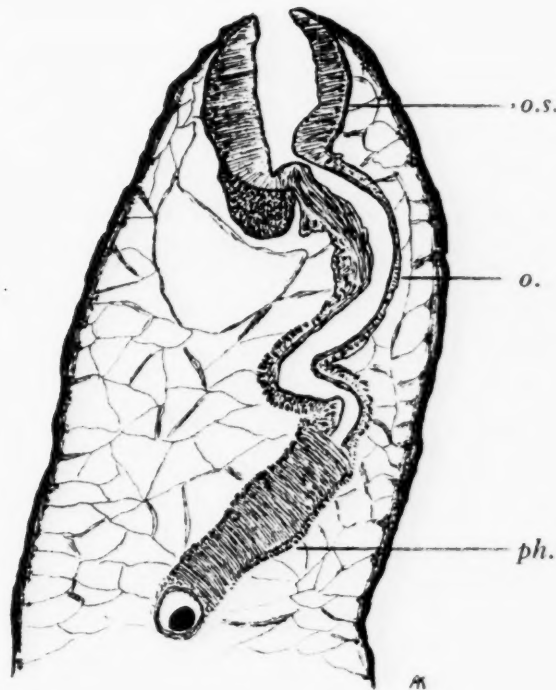


FIG. 4

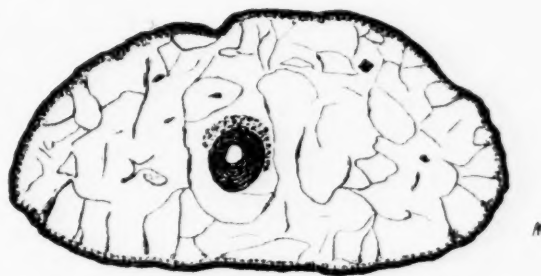


FIG. 5

FIG. 3. *Chiorchis purvisi* sp. nov. Lateral view. (\times about 18.)

FIG. 4. *Chiorchis purvisi* sp. nov. Sagittal section, showing oesophagus and pharynx. (\times about 42.)

FIG. 5. *Chiorchis purvisi* sp. nov. Transverse section, showing musculature of pharynx. (\times about 30.)

(Lettering as in figs. 1 and 2.)

HISTORY OF CLASSIFICATION

Whatever characters are ascribed to the class Trematoda, it nevertheless remains a fact that it is difficult, if not impossible, to define the limits of the group. The classification of the trematodes into the Monogenea and the Digenea dates back to Van Beneden (1858). The Monogenea are accepted as being almost entirely ectoparasitic, although a few are known to occur in the excretory and respiratory systems of certain hosts. They are presumed to have a direct life-history. It is, however, true to say that in only a few cases is the life-history known. Where it is known, it is direct, but it might easily be true that in other species alternation of generations may occur, and this is especially so in the case of those Monogenea which are internal parasites. Morphologically

they are different from the Digenea in the possession of well-developed organs for attachment, these being situated usually at the posterior extremity, and consisting of suckers, chitinous hooks or anchors. The paired excretory pores are placed anteriorly.

In the Digenea, the life-history shows alternation of generations in all cases where it is known. Chitinous organs of attachment are absent; the excretory pore is single and usually situated posteriorly; and there are typically two suckers only, although in a few cases a third may be present. The subclass Digenea is divided into two orders, namely, *Gasterostomata*, in which the mouth is on the mid-ventral surface, and is not surrounded by a sucker, the intestine being a simple sac; and *Prosostomata*, in which the mouth is surrounded by a sucker, and is situated anteriorly. It is in the latter order that the bulk of the parasites of medical and veterinary importance occur, including the amphistomes.

We assume that the group known as amphistomes were originally separated from other digenetic trematodes on account of the ventral sucker being placed at the posterior extremity.

Fischoeder (1901-1903) divided the paramphistomes (= amphistomes) into two subfamilies, viz. :—

1. *Paramphistominae*, including those forms without paired oral diverticula, in which the testes are lobed, and a cirrus pouch is absent. The genus *Stephanopharynx* was included in this subfamily, although it possesses a *single* oral diverticulum.

2. *Cladorchinae*, in which the oral diverticula are paired, testes ramified, and a cirrus pouch is present.

Stiles and Goldberger (1910) raised the amphistomes to superfamily status (*Amphistomoidea*); they created two new families, and retained Fischoeder's *Paramphistomidae* emended. The characters of the three families were :—

1. GASTRODISCIDAE. 'Body discoidal, divided into an anterior and a posterior portion.' Three genera.

2. GASTROTHYLACIDAE. This included all forms having a ventral pouch; with one subfamily, *Gastrothylacinae*, containing 4 genera, one of which—*Carmyrius*—was divided into 5 subgenera.

3. PARAMPHISTOMIDAE included worms without a ventral pouch, and in which the body is not divided into two parts. This family contained 4 subfamilies as follows :—

- (i) *Paramphistominae*. Oral diverticula absent; 2 genera.

- (ii) *Stephanopharynginae*. With one oral diverticulum; 1 genus.

- (iii) *Cladorchinae*. With two oral diverticula; 9 genera.

- (iv) *Diplodiscinae*. With two oral diverticula, but having the excretory system branching radially in the acetabulum. The testes coalesce in the adult; 3 genera.

Stunkard (1925) classified the group as follows :—

- Subfamily 1. *Paramphistominae*. Without oral diverticula.
 2. *Cladorchinae*. With two oral diverticula.
 3. *Schizamphistominae*. With two oral diverticula. Separated on account of the peculiar lymphatic and excretory systems.
 4. *Gastrothylacinae*. Having a ventral pouch, but no oral diverticula. No other subfamily possesses a ventral pouch.
 5. *Zygocotylinae*. With two oral diverticula. Posterior sucker divided, or with overhanging lip; oral sucker subterminal, ventral; openings of the male and female ducts separate. Stunkard refers to the possible relationship of this subfamily to *Diplodiscinae*; but in the latter there is a cirrus sac and both suckers are terminal.
 6. *Brumptinae*. With two oral diverticula; the posterior extremity is divided into two, each part containing a large vitelline gland.
 7. *Balanorchinae*. With two oral diverticula. Ovary in front of the testes.
 8. *Diplodiscinae*. With two oral diverticula. Characterized by the acetabulum, which either bears an accessory sucker, or is divided, or is broader than the body. Stunkard rightly stated that the characters of this subfamily are ill-defined.
 9. *Gastrodiscinae*. Body divided into an anterior and posterior portion, one of which is usually flattened. With 3 genera, viz., *Gastrodiscus*, *Gastrodiscoides* and *Homalogaster*.

The subfamily *Stephanopharynginae* Stiles and Goldberger, 1910, was not accepted by Stunkard, but he recognized the genus *Stephanopharynx*, which he placed in the *Paramphistominae*.

Poche (1925) created a super-superfamily *Paramphistomida*, in which he recognized three families, namely, *Paramphistomidae* Fiscoeder, 1901, *Dissotrematidae* Goto and Matsudaira, 1918, and *Angiodictyidae* Looss, 1902. In the *Paramphistomidae* he included the families *Gastrodiscidae* Stiles and Goldberger, 1910, and *Gastrothylacidae* Stiles and Goldberger, 1910. He retained the family *Angiodictyidae* on account of the fact that all species possessed an excretory and lymphatic system resembling that of amphistomes, but more highly differentiated. It is to be noted, however, that species of this family do not possess an acetabulum; nevertheless Poche retained them in the *Paramphistomida*.

Nicoll (1915) described, amongst others, two parasites which he named *Opistholebes amplicoelus* and *Gyliauchen tarachodes* respectively. Goto and Matsudaira in 1918 described a species which they named *Dissotrema papillatum* gen. nov., sp. nov. In 1919 Goto published a paper in which he pointed out that the genus *Dissotrema* was synonymous with Nicoll's *Gyliauchen*. Fukui

(1929) erected a subfamily, *Gyliaucheninae*, containing two species only, namely, Nicoll's species and *Dissotrema papillatum* (Goto and Matsudaira, 1918).

Ozaki (1933) raised the subfamily *Gyliaucheninae* to the status of family. In these circumstances it is difficult to understand why Poche retained the family *Dissotrematidae* Goto and Matsudaira, 1918.

The genera *Gyliauchen* and *Opistholebes* appear to us to be so closely related that, along with four other genera, we have placed them in the subfamily *Opistholebetinae* Fukui, 1929. Poche does not mention the genus *Opistholebes*, but, as it was the first of the two genera noted above which were described by Nicoll, it is, we consider, right and proper that it should become the subfamily name.

Fuhrmann (1928) follows Stunkard, but does not accept the subfamily *Gastrodiscinae*; he considers five other genera as being of uncertain position.

Fukui (1929) proposed a most elaborate scheme of classification, summarized below.

He divided the *Amphistomata* into 4 families, differentiated as follows :—

KEY TO FAMILIES

- | | | |
|----|---|---------------------------|
| 1. | { Genital pore in the posterior part of body. | <i>Opisthoporidae</i> |
| | { Genital pore in the anterior part of body. | 2 |
| 2. | { Excretory system with rosette-shaped diverticula, a sac-like portion and eight main canals; without acetabulum. | <i>Angiodictyidae</i> |
| | { Excretory system without rosette-shaped diverticula, vesicle simple; with acetabulum. | 3 |
| 3. | { Ovary usually post-testicular, never anterior to testes; without seminal receptacle. | <i>Paramphistomatidae</i> |
| | { Ovary apparently pre-testicular; with seminal receptacle. | <i>Opistholebetidae</i> |

Family (1) *Opisthoporidae* Fukui, 1929, contains 1 genus only.

Family (2) *Angiodictyidae* Looss, 1902, contains 5 genera.

Family (3) *Opistholebetidae* Fukui, 1929, contains 2 subfamilies, differentiated as follows :—

KEY TO SUBFAMILIES OF *OPISTHOLEBETIDAE*

Oesophagus very long and convoluted, caeca reaching to the middle of the body, vitellaria anterior, cirrus pouch not enclosing the vesicula seminalis, body cylindrical.

Gyliaucheninae

Oesophagus very short and not convoluted, caeca reaching to posterior end of the body, vitellaria lateral, extending through nearly the whole body length, cirrus pouch enclosing the vesicula seminalis, body ovoid.

Opistholebetinae

Family (4) *Paramphistomidae*, which he classified as indicated below.

KEY TO SUBFAMILIES

- | | | |
|----|---|-----------------------------|
| 1. | { With hermaphroditic pouch. | <i>Brumptinae</i> |
| | { With cirrus pouch | 2 |
| | { Without cirrus pouch. | 4 |
| 2. | { Without genital papilla ; cirrus pouch special, testes markedly posterior. | <i>Balanorchinae</i> |
| | { Cirrus pouch elongate and spindle-shaped, very muscular. | <i>Pfenderinae</i> |
| | { Cirrus pouch not so elongate or muscular. | 3 |
| 3. | { Testes usually spherical, smooth, sometimes one. Acetabulum usually with central sucker-like projection, or divided into two parts. | <i>Diplodiscinae</i> |
| | { Testes usually lobed, acetabulum simple ; without genital sucker. | <i>Schizamphistomatinae</i> |
| | { Testes branched or lobed, acetabulum simple, papillated or with appendage ; genital sucker present or absent. | <i>Cladorchinae</i> |
| 4. | { Body divided into two parts. | <i>Gastrodiscinae</i> |
| | { Body not divided into two parts. | 4 |
| 5. | { Oral sucker ventral, acetabulum divided into two parts and with paired appendages. | <i>Zygocotylinae</i> |
| | { Testes smooth, spherical ; with genital sucker. | <i>Dadayinae</i> |
| | { Testes various ; with or without genital sucker. | <i>Paramphistomatinae</i> |

The subfamily *Paramphistomatinae* he divided into 3 tribes, viz. :—

KEY TO TRIBES

- | | |
|---------------------------------|----------------------------|
| Without oral evagination. | <i>Paramphistomatinea</i> |
| With a single oral evagination. | <i>Stephanopharynginea</i> |
| With paired oral evaginations. | <i>Pseudodiscinea</i> |

Fukui gave a most useful and extensive revision, not only of the classification, but of the morphology, anatomy, and even the histology of group, which he also re-classified. He divided them into 4 families, 14 subfamilies, 30 genera, and 14 subgenera. Amongst the numerous morphological characters which he selects in the differentiation of his families, subfamilies and genera, there are many which, in our experience, are of very doubtful value. Amongst these characters we may mention the following :—

1. *The excretory system.* The elucidation of this system is a matter of great difficulty, usually requiring sections.

2. *Hermaphroditic pouch.* This may be so small as to be unrecognizable.

3. *Genital papillae*. Maplestone called attention to the inconstancy of these organs.

4. *Cirrus pouch*. In his key to the subfamilies of the *Paramphistomidae*, Fukui states that the pouch is 'elongate and spindle-shaped, very muscular,' whilst in seven other subfamilies the pouch is 'not so elongate or muscular.' It is our opinion that the line of demarcation between these two so-called opposite characters cannot be fixed.

5. *The testes*. The families are differentiated according to whether the testes are 'usually spherical,' 'usually lobed,' or 'branched or lobed.' We shall later call attention to the fact that the shape of the testis varies within such wide limits that we have found it impossible to place certain appearances of these organs in any of the categories mentioned by Fukui.

It is neither our wish nor our intention to criticize the systematic scheme proposed by this author; all that we desire is to call attention to the fact that on many occasions we have found it impossible to say whether, for instance, the testis was lobed, the hermaphroditic duct long or short, the cirrus pouch not so long or muscular, and so on. Therefore, we think that these characters have very little value, unless expressed in different terms.

Travassos (1934) accepts all Fukui's subfamilies except *Gastrodiscinae*, which he raises to family status. He also retains the old subfamily *Stephanopharynginae* for the genus of that name. Further, to the superfamily *Paramphistomoidea* Stiles and Goldberger, 1910, he ascribed, amongst others, the following characters:—'The acetabulum is terminal, sub-terminal, or absent.' Fukui (1929) had previously included in the amphistomes the family *Angiodictyidae*, originally included in the monostomes, but regarded by Odhner as amphistomes which had lost their acetabulum. He erected 6 families, viz. :—

A. PARAMPHISTOMIDAE. Oral diverticula, ventral pouch and pharynx present or absent. Lymphatic system with one or three pairs of longitudinal trunks. He recognized 9 subfamilies, with the following characters :—

1. *Paramphistominae* Fischöeder, 1901. Oral diverticula and pharynx absent. Acetabulum prominent. Testes in front of the ovary. Vitelline glands extra-caecal. Lymphatic system consists of one pair of longitudinal trunks.

2. *Gastrothylacinae* Stiles and Goldberger, 1910. Ventral pouch present. Oral diverticula and pharynx absent. Genital opening close to that of the ventral pouch. Cirrus pouch absent. No genital sucker. Lymphatic system consists of one pair of longitudinal trunks. This is the only subfamily in which a ventral pouch is present.

3. *Stephanopharynginae* Stiles and Goldberger, 1910. One oral diverticulum present. Pharynx absent. Gut caeca sinuous, and extending as far as the acetabular zone. Genital sucker absent, but genital pore surrounded by a well-developed musculature. Testes in front of the ovary. Laurer's canal in the mid-line.

4. *Cladorchinae* Fiscoeder, 1901. Paired oral diverticula present. Gut caeca reaching, or extending beyond, the acetabular zone. Ovary posterior to the testes. Lymphatic system consists of three pairs of longitudinal trunks.

5. *Zygocotylinae* Ward, 1918. Oral diverticula and pharynx present. Genital sucker and cirrus pouch absent. Acetabulum with two lateral papillae. Lymphatic system consists of three pairs of longitudinal trunks, one dorsal and two ventral.

6. *Balanorchinae* Stunkard, 1925. Oral diverticula, genital sucker and cirrus pouch present. Pharynx absent. Ovary anterior to the testes.

7. *Kalitrematinae* Travassos, 1933. Anterior extremity dilated and bearing a collar; posterior end divided into two semicircular lobes. Oral diverticula and genital sucker present. Pharynx and cirrus pouch absent. Testes lobed, extra-caecal, on a level with the bifurcation. Ovary posterior to the testes, on a level with the end of the gut caeca. The latter terminate far short of the acetabulum.

8. *Diplodiscinae* Cohn, 1904. Genital sucker absent. Oral diverticula and pharynx present. Acetabulum large, either with an accessory sucker, or divided into two by a muscular constriction. Testes equatorial, fused, rarely separated. Ovary posterior to the testes.

9. *Brumptinae* Stunkard, 1925. Posterior extremity divided into two, each part containing a large vitelline gland. Pharynx absent. Oral diverticula and genital sucker present. Ovary posterior to the testes. Cirrus pouch well-developed. Lymphatic system complex.

B. GASTRODISCIDAE Stiles and Goldberger, 1910. Body divided into an anterior and posterior part, the ventral surface of the latter transformed into an organ of fixation, and provided with papillae. Acetabulum relatively small. Testes, ovary and uterus intra-caecal. Oral diverticula present. Pharynx absent or rudimentary. Genital pore at the junction of the anterior and posterior parts. With 3 genera, viz., *Gastrodiscus*, *Gastrodiscoides* and *Homalogaster*.

C. OPISTHOLEBETIDAE Fukui, 1929. Oral diverticula absent. Oesophagus very short. Pharynx present. Caeca very large. Testes posterior to the ovary. Lymphatic system? The family contains 1 genus only.

D. GYLIAUCHENIDAE Ozaki, 1933. No oral diverticula. Oesophagus long and sinuous. Pharynx present. Caeca short and stout. Testes posterior to the ovary. Lymphatic system? Cirrus pouch present or absent. The family contains 3 genera.

E. CEPHALOPORIDAE Travassos, 1934. Cuticle armed with spines. Acetabulum small and subterminal. Pharynx immediately behind the oral sucker. Oesophagus long. Genital pore lateral, opening at the anterior extremity. Ovary in front of the testes. Uterus behind the ovary. Two genera in the family.

F. MICROSCAPHIDIIDAE Travassos, 1922. Acetabulum absent. Oral diverticula

absent or rudimentary. Pharynx present or absent. Testes anterior to the ovary. Vitelline glands intra- or extra-caecal. Lymphatic system with two, three or four pairs of longitudinal trunks. The family contains 8 genera.

From the above it will be seen that Travassos recognised 6 families, 9 subfamilies, 55 genera, and 2 subgenera; the total number of species dealt with in his synopsis is about 105. In such a prolixity of genera, many of which are very closely related (we think identical), we have found great difficulty in ascertaining exactly what are the characters of the various genera detailed.

PRINCIPAL CHARACTERS USED IN CLASSIFICATION

Most investigators accept as amphistomes those digenetic trematodes which possess an acetabulum (sucker) at each extremity of the body. Fukui (1929), however, includes in the amphistomes the family *Angiodictyidae* Looss, 1902. Originally placed in the monostomes, these were later considered by Odhner to be amphistomes which had lost their acetabulum. Both Fukui (1929) and Travassos (1934) include them in the amphistomes. Travassos (1934) also includes the family *Microscaphidiidae* Travassos, 1922 (= *Microscaphinae* Looss, 1899) in the amphistomes, and, as noted elsewhere, states that an acetabulum may be absent. Some authors refer to the group as a family, whilst others have raised them to the status of a superfamily.

We now propose to refer briefly to the principal characters which various authors have utilized in the differentiation of families and subfamilies.

Fischoeder called the group a family, and differentiated two subfamilies principally by one character: *Paramphistominae*, forms without paired oral diverticula, and *Cladorchinae*, forms with paired oral diverticula.

Stiles and Goldberger (1910) elevated the family to a superfamily, and recognized 3 families, (1) *Paramphistomidae*, without ventral pouch, (2) *Gastrothylacidae*, with a ventral pouch, and (3) *Gastrodiscidae*, without a ventral pouch, but having the body divided into two parts.

Stunkard (1917) split the family into 9 subfamilies, which he differentiated by the possession or otherwise of some of the following characters:—

- | | |
|--|---------------------------|
| 1. Oral diverticula. | |
| 2. Peculiar lymphatic and excretory systems. | <i>Schizamphistominae</i> |
| 3. Ventral pouch present. | <i>Gastrothylacinae</i> |
| 4. Acetabulum divided, and oral sucker subterminal | <i>Zygocotylinae</i> |
| 5. Ovary in front of the testes. | <i>Balanorchinae</i> |
| 6. Acetabulum divided, or broader than body, or bearing an accessory sucker. | <i>Diplodiscinae</i> |
| 7. Body divided into two parts. | <i>Gastrodiscinae</i> |
| 8. Posterior extremity divided into two, each part with a vitelline gland. | <i>Brumptinae</i> |

Maplestone (1923) considered that the group contained 3 families, separated as shown below :—

- | | |
|---|-------------------------|
| 1. Body divided into an anterior and a posterior portion. | <i>Gastrodiscidae</i> |
| 2. Ventral pouch absent, body not divided. | <i>Paramphistomidae</i> |
| 3. Ventral pouch present, body not divided. | <i>Gastrothylacidae</i> |

Subfamilies were differentiated as follows :—

- | | |
|---|----------------------------|
| <i>Paramphistomidae</i> . 1. Oral diverticula absent. | <i>Paramphistominae</i> |
| 2. Oral diverticula double. | <i>Cladorchinae</i> |
| 3. Oral diverticulum single. | <i>Stephanopharynginae</i> |

The other two families contain no subfamilies.

Fukui (1929) divides the amphistomes into 4 families, utilizing the following as the principal characters :—

- | | |
|--------------------------------------|---------------------------|
| 1. Genital pore posterior. | <i>Opisthoporidae</i> |
| 2. Elaborate excretory system. | <i>Angiodictyidae</i> |
| 3. Position of ovary. | <i>Paramphistomatidae</i> |
| 4. Presence of a seminal receptacle. | <i>Opistholebetidae</i> |

The subfamilies were differentiated with reference to the following characters :—

1. Hermaphroditic pouch.
2. Cirrus pouch.
3. Genital papillae.
4. Position and shape of the testes.
5. Acetabulum with central sucker-like projection, or divided.
6. Genital sucker.
7. Body divided into two parts.
8. Position of the oral sucker.

It is to be noted that Fukui utilizes characters in his family distinctions which had not previously been used by other authors. Oral diverticula are considered by him to be of tribal value, whilst a ventral pouch is used simply to differentiate a genus.

Travassos (1934) divided the amphistomes into 6 families, the principal points of differentiation being :—

- | | |
|---|----------------------------|
| 1. Body divided into anterior and posterior parts. | <i>Gastrodiscidae</i> |
| 2. Spines on body; genital pore anterior and lateral. | <i>Cephaloporidae</i> |
| 3. Testes in front of the ovary. | { <i>Gastrodiscidae</i> |
| | { <i>Microscaphidiidae</i> |
| | { <i>Paramphistomidae</i> |
| 4. Absence of acetabulum | <i>Microscaphidiidae</i> |

5. Oral diverticula, ventral pouch and pharynx present or absent.

6. Length of oesophagus.

Paramphistomidae
 { *Opistholebetidae*
Gyliauchenidae

Of his families, the only one in which there are subfamilies is the *Paramphistomidae*, and this is divided into 9, differentiated on the following points:—

1. Ventral pouch present.

2. Single oral diverticulum

3. Paired oral diverticula.

4. Testes behind the ovary.

5. Collar round the head; posterior extremity divided.

6. Posterior end divided into two, each part containing vitellaria.

7. Acetabulum modified.

Gastrothylacinae

Stephanopharynginae

Cladorchinae, *Zygocotylinae*, *Balanorchinae*,
Diplodiscinae, *Brumptinae*, *Kalitrematinae*

Balanorchinae

Kalitrematinae

Brumptinae

Diplodiscinae

REMARKS ON THE ABOVE CHARACTERS

Every systematist is painfully aware of the fact that nature is very plastic, and that organs frequently vary within fairly wide limits; it is this circumstance which makes his work so difficult, and often so unreliable. Because of this, it is the considered judgment of the authors that no scheme, however carefully thought out, can ever be entirely satisfactory. It is probably true to say that very few systematists, working on any group of parasites, agree in the schemes of classification which they evolve as a result of their investigations. A study of the preceding pages is a striking proof of this fact.

The results of the different classifications are very involved and of such a nature that it is extremely difficult for a beginner to ascertain even the subfamily to which his specimen may belong. This was our experience when we attempted to name the species of amphistome which is described in the earlier part of this paper. A careful and prolonged study of the literature (which is very extensive), and some practical knowledge of a considerable number of species, led us to the conclusion that it ought to be possible to produce a simpler scheme of classification, which would enable any worker to relegate his species to its proper genus. Any, and all, schemes of classification should aim at simplicity, utility and practicability, and should, in our opinion, be based on characters which can be ascertained without the necessity of serially sectioning the parasite in question. With reference to the latter point, it will be obvious that, in a small collection of parasites from one animal, it may easily happen that the parasite sectioned may be quite different from the rest, and thus wrong results may be arrived at.

We are convinced that some simplification in the case of the amphistomes is possible, and we have, with considerable reluctance, ventured to produce the scheme set forth in this paper.

It is quite obvious that the result of any scheme of classification depends entirely on the characters, or combination of characters, which are selected as a basis. Thus, if a number of parasites are placed in one group because they all possess a muscular pharynx, they would not all be contained in the same group if the classification was based on, say, the form and position of the testes. This condition would apply to almost every organ of the body. In this connection, we have thought it useful to review briefly the variations in the different organs which have been, and are still, used in the classification of the amphistomes.

SHAPE OF THE BODY

The characters ascribed to the family *Gastrodiscidae* Stiles and Goldberger, 1910 (= *Gastrodiscinae* Monticelli, 1892), are as follows :—

The body is rather discoidal, divided by a transverse constriction into cephalic and caudal portions; ventral pouch absent, venter with many large papillae. Acetabulum ventral at caudal end. This family contains 3 genera, namely :—

1. *Gastrodiscus* Leuckart, 1877. There are 2 species belonging to this genus. In each case the body is distinctly divided into an anterior, somewhat conical, part, and a posterior circular part, whose ventral surface is studded with rows of large papillae. When viewed dorsally, the parasite resembles a tortoise in shape.

2. *Gastrodiscoides* Leiper, 1913. The body is divided into the same two parts as in *Gastrodiscus*, but the ventral surface of the posterior part does not bear papillae. The genus contains a single species, namely, *Gastrodiscoides hominis* (Lewis and McConnell, 1876). Baylis (1929) states that 'the validity of the genus is still *sub judice*, the distinctions having been based on characters that appear to be subject to great variation in worms preserved in different states of contraction.'

3. *Homalogaster* Poirier, 1883. The genus contains a single species only. Its characters are quite different from those of the two preceding genera. In the first place, it is the anterior part of the body which bears, ventrally, numerous large papillae, each of which appears to us to terminate in a minute sucker. The so-called posterior part consists of the acetabulum only, there being no clear division of the body into two parts, unless one considers that the acetabulum is one part, and the rest of the body is another part. The presumed division of the body into two parts is no more distinct than is the case in *Diplodiscus subclavatus* (Goeze, 1782), *Paramphistomum formosanum* Fukui, 1929, or in some other species which could be cited.

It will be clear that *Homalogaster* is totally different from the other two genera in two points of major importance, namely, the body is not divided into two, and the papillae are borne on the ventral surface of the whole body except the acetabulum.

As far as we are aware, the only genera in which the body appears to be definitely and permanently divided into two are *Gastrodiscus* and *Gastrodiscoides*. We retain these genera in the subfamily *Gastrodiscinae*, and we place the genus *Homalogaster* in the subfamily *Cladorchinae*. In the *Brumptinae*, the posterior part of the body is divided into two by a deep sulcus, each half containing a large vitelline gland. In the *Kalitrematinae*, the posterior part of the body is also divided into two, but in this case the two halves do not contain vitellaria.

BODY COVERED WITH SPINES

One of the principal characters of the family *Cephaloporidae* is the fact that the body is covered with spines. We have reason for believing that such spines are in all probability deciduous. In young parasites they are probably present; in old parasites, probably entirely absent; and in other specimens they may be present in small patches here and there. If our assumption is correct, the value of a spiny cuticle is very doubtful, as the presence or absence of such spines would depend largely on the age of the parasite. Even when present in the fresh specimens they may fall from the skin as a result of preservation.

PAPILLAE ON BODY

We have noted above that the presence of papillae on the ventral surface of *Gastrodiscus*, and their absence in *Gastrodiscoides*, is, apart from the shape of the body, one of the principal distinctions between these two genera.

Papillae are present on the ventral surface of *Homalogaster*, and it is this character which separates this genus from others belonging to the subfamily *Cladorchinae*. Minute oral papillae are found in practically all species in the subfamily *Paramphistominae* Fischöeder, 1901, and also in many species of *Cladorchinae*. In *Paramphistomum formosanum* the entire ventral surface of the body is covered with minute papillae. In this case, however, the papillae have only specific importance.

We have referred previously to *Zygocotyle* in which the posterior sucker bears lateral papillae; in a number of other species, such as *Pfenderius papillatus*, minute papillae are found covering the surface of the acetabulum.

GENITAL PORE

As far as we are aware, in amphistomes there is a single genital pore. In the family *Cephaloporidae* Travassos, 1934, this is situated quite anteriorly and laterally. Genera and species of this family can, therefore, be relegated to this group by locating the position of the pore. In the *Opisthoporidae* Fukui, 1929, it is situated posteriorly. In all other amphistomes it is usually near the bifurcation of the caeca. The genital pore in some species is surrounded by a sucker, and this character is of considerable systematic value. In other

species the aperture is surrounded by muscular tissue (possibly sphincter), which appears to be of less systematic importance. The pore, however, is never lateral except in *Cephaloporidae*. Great variations occur with reference to the openings of the vas deferens and the uterus (vagina). In most cases the genital pore leads into a depression or cavity, which we may consider as the genital atrium. At the base of this atrium there is usually a genital papilla, and it is on this papilla that the genital ducts open. In some cases the vas deferens and the uterus open separately, but quite close together. In other cases the terminal parts of the vas deferens and the uterus unite into a common duct called the ductus hermaphroditicus. This may be comparatively long or short. When short it is almost impossible to say definitely whether it is actually present or not.

Further, in some species a cirrus pouch is present, whilst in others it is absent. In still others it is so small that it may be impossible to decide whether it is definitely present or absent.

Whilst it is clear that the anterior and lateral position of the pore is a character which can be easily determined and is of great value, the points noted above are often extremely difficult to determine unless the worm is serially sectioned. At the most, these points are of minor systematic value.

POSITION OF THE ORAL SUCKER

In the subfamily *Zygocotylinae*, Stunkard, amongst other characters which he gives, states that in this subfamily the oral sucker is subterminal. In our opinion this character is of very doubtful value, as it is possible for the sucker to be terminal, but to appear subventral as a result of muscular contraction.

POSTERIOR SUCKER (ACETABULUM)

Usually the acetabulum is terminal, but in some genera it is either subterminal or ventral. When markedly ventral, the character is of value. In the genus *Megalodiscus* (= *Diplodiscus*) the acetabulum is much broader than the body; in the genus *Catadiscus* it is, to all intents and purposes, divided into two by a lateral constriction; whilst in the genus *Opisthodiscus* it bears a small, central, accessory sucker. In the *Zygocotylinae* (*Zygocotyle* = *Stunkardia*) it bears a pair of postero-lateral papillae.

ORAL DIVERTICULA

These are absent in the *Paramphistomidae* and the *Gastrothylacidae*; they are present in the *Cladorchidae*, and are paired in all species except *Stephanopharynx*. Fukui calls attention to the fact that this organ is developed in different degrees in various species, and Stunkard (1925) writes as follows: 'In the Cladorchinae, however, there are several species in which the sacs are so reduced that they are enclosed within the muscular wall of the sucker and almost indistinguishable. Within the group there is every gradation from this rudimentary or vestigial

condition to large sacs exceeding the sucker in size. Slightly more reduction would give the condition characteristic of *Paramphistomum* and the median migration and fusion of the sacs would produce the single median evagination of *Stephanopharynx*. It would seem therefore that the number of oral sacs, while obviously a convenient means of separating forms, is not of fundamental and primary importance in the formulation of a natural system of classification. The erection of a subfamily to contain *Stephanopharynx* serves no useful purpose; the presence of a single oral sac in this form is probably of no more significance than the presence of a genital sucker in *Cotylophoron* and since in other respects these genera are so similar to *Paramphistomum*, they should for the present at least be included with it in the subfamily Paramphistominae.'

The question raised by Stunkard relates to the relative value of various organs from the point of view of classification, the suggestion being that the presence of a single diverticulum in the one case serves to identify the subfamily, whilst the presence of a genital sucker is only one of many characters ascribed to certain other genera in other subfamilies. In any case, the oral diverticula, like every other part of the body, are subject to great variations, and it is this fact alone which makes the work of the systematist difficult.

VENTRAL POUCH

Only in one family is a ventral pouch present, namely, in the *Gastrothylacidae*. In all cases it seems to be well developed and not to share in that great variation exhibited by most other organs of the body.

PHARYNX

The occurrence or otherwise of a muscular pharynx would appear to be subject to less variation than is the case with many other organs of the body. In those amphistomes with a pharynx it is usually situated at the posterior end of the oesophagus. In *Cephaloporus*, however, it is placed at the anterior end. Its presence or absence is an extremely useful character in the separation of the various genera in the *Cladorchinae*.

LENGTH OF THE OESOPHAGUS

The oesophagus varies in length within somewhat narrow limits, usually being not more than twice the length of the oral sucker. In the genus *Gyiliauchen*, however, it is a long coiled tube, almost ten times the length of the sucker.

THE GUT CAECA

Great variations are to be observed in these organs: in some the caeca are short, in others they extend to the posterior extremity of the body; in some they are straight, in others they are markedly sinuous; and in some instances they are narrow, in others they are broad. These varying conditions are extremely useful in the differentiation of different species.

EXCRETORY AND LYMPHATIC SYSTEMS

Very little is known regarding these systems in most species of the group, and, in fact, to determine the distribution of the vessels in the body would in most cases necessitate the parasite being sectioned serially. If for no other reason, we regard it as impossible to accept Looss's suggestion that the classification of the group should be based on these systems. In this connection Maplestone states that : ' It is probable that Looss' suggestion to classify the members of the group on the minute anatomy of the lymphatic, excretory, and copulatory systems is sound, but it appears to be too complicated for practical purposes.'

TESTES

Fukui (1929) gives a very useful diagram showing the position which one testis may bear to the other, and also of the relation of the testes to the ovary. He points out, however, that the arrangements are ' more or less constant in the species, so it may be used for diagnosis to some extent.' It is quite clear that these relationships vary within wide limits in all the subfamilies as at present accepted. As far as we are aware, it is only in the *Balanorchinae* and the *Opistholebetinae* that the ovary is situated in front of the testes. These two subfamilies are separated because in the former there are paired oral diverticula whereas in the latter these organs are absent. This means that in our classification the presence or absence of oral diverticula is of family importance, whereas the anterior position of the ovary is a subfamily character. We are not prepared to argue that these characters have a correspondingly different relative value.

We have, on one or two occasions, been unable to determine whether the testis was lobed or not ; we have seen specimens in which one testis had a perfectly smooth surface, and the other showed distinct lobulations, within the one animal. We are, therefore, not impressed with the character of ' lobulations,' unless it is known to be constant and the lobulations such that it is possible to see them, as in *Chiorchis* and *Cladorchis*.

In some cases, the testes appear to be fused together, as in species of *Diplodiscinae* and *Paracotylinae*.

We have experienced great difficulty in determining, in some cases, whether a testis was ' ramified ' or deeply lobed. In *Chiorchis* each testis is divided into four prominent lobes, which can be considered as ' ramified ' ; the same is the case in *Travassosinia* and *Nematophila*. In the latter it is impossible to decide whether the testes are lobed or ' ramified ' ; they appear to us dendritic. For this reason the use of the words ' lobed ' and ' ramified ' are frequently misleading. We are familiar with five main appearances of the testes : (1) A solid body more or less round, either with an even surface, or with very shallow sulci, as in *Alassostoma*. (2) Testes, as in *Chiorchis fabaceus*, which are made up strictly of digitate processes. (3) Testes like those of *Nematophila*, which we consider to be dendritic. (4) Testes like those of *Travassosinia*, which consist

of a solid body with lobulated margins. And lastly (5), testes like those of *Cladorchis* (*Stichorchis*) and *Taxorchis*, which appear to us to consist of a coiled tubular structure; parts of the coil may fuse here and there into one or more small solid areas; the appearance, nevertheless, is that of a coiled tube.

In a general way, we may state that, in the genus *Cladorchis*, the testes present the appearance of an irregularly convoluted tube, whereas in the rest of the amphistomes they are mostly solid bodies, showing varying degrees of shallow or deep lobulations. In nearly all cases they are intra-caecal, except in certain species of *Opistholebetinae*.

OVARY

This is usually situated behind the testes. It lies between the testes in quite a number of genera which are not otherwise closely related, and in front of the testes in those cases noted above.

LAURER'S CANAL

It is by no means easy to find this duct in fleshy worms like the amphistomes. When the parasite is gravid, it is almost always necessary serially to section the worm in order to locate it. For this reason we are not impressed with its importance as a diagnostic character, except perhaps in the differentiation of species.

VITELLINE GLANDS

These are nearly always extra-caecal. The acini may be separate or may consist of a number of small glands. In *Brumptia* the posterior part of the body is bifid, each half containing a vitelline gland. In other cases it occupies varying lengths of the lateral margins, sometimes being confined more particularly to the posterior part of the body. In a few species the gland is intra-caecal, as, for instance, in *Ophioxenos* Sumwalt, 1926. In yet other cases part of it is intra- and part extra-caecal on both sides, as in *Diplodiscus subclavatus* (Goeze, 1782). Realizing that in almost all amphistomes the vitelline glands are extra-caecal, it might be useful to relegate to specific genera those forms in which (1) the glands were entirely intra-caecal and (2) those in which they overlapped.

EGGS

We note that in the descriptions of the majority of amphistomes nothing is said about the egg. We do not think that, even if the details were available, they would provide many important additions which would be of use to the systematist; but it would be interesting to know the size of the eggs and the nature of the contents—that is, whether the egg contained an ovum, a miracidium, or some intermediate stage. We can conceive that an extra large-sized egg might be diagnostic, and so might an egg which contained an ovum. We think it desirable, whenever possible, to describe the egg found in the faeces of the animal from which the adult worm was obtained.

PROPOSED CLASSIFICATION

Superfamily PARAMPHISTOMOIDEA Stiles and Goldberger, 1910

The superfamily includes all trematodes in which there is a sucker at each extremity; they are believed to be digenetic. We recognize 3 families :—

FAMILY A. PARAMPHISTOMIDAE FISCHOEDER, 1901

Amphistomes in which the oral diverticula and ventral pouch are absent. The family is divided into 3 subfamilies :—

KEY TO SUBFAMILIES

- | | |
|------------------------------------|----------------------------|
| With one testis. | <i>Paracotylinae</i> n.sf. |
| With two testes. | 1 |
| 1. { Testes posterior to ovary. | <i>Opistholebetinae</i> |
| { Testes never posterior to ovary. | <i>Paramphistominae</i> |

Subfamily 1. *Paramphistominae* Fischoeder, 1901. Two testes nearly always anterior, but never posterior, to the ovary. Two genera only are included in this subfamily, viz.:—

- (i) *Paramphistomum* Fischoeder, 1901. Pharynx and genital sucker absent.
 (ii) *Cotylophoron* Stiles and Goldberger, 1910. Pharynx and genital sucker present.

Under the genus *Paramphistomum* we have included *Paramphistomum aspidonectes* McCallum, 1917. Fukui (1929) erected a new family, *Opisthoporidae*, to include this single species, on account of the posterior position of the genital pore and the peculiar arrangement of the vitellaria.

Subfamily 2. *Opistholebetinae* Fukui, 1929. With two testes posterior to the ovary. Five genera have been placed in this family; they can be differentiated by the following key :—

- | | |
|---|----------------------|
| Genital pore quite anterior and lateral. Pharynx at oral end of oesophagus. Body covered with spines. | <i>Cephaloporus</i> |
| Genital pore median in the region of the bifurcation of caeca. Pharynx, if present, at posterior end of oesophagus. Body not covered with spines. | 1 |
| 1. { Testes and ovary posterior to acetabulum. | <i>Paragyliuchen</i> |
| { Testes and ovary not posterior to acetabulum. | 2 |
| 2. { Testes in same transverse line as acetabulum. | <i>Telotrema</i> |
| { Testes not in same transverse line as acetabulum | 3 |
| 3. Oesophagus very short, about the length of the oral sucker. Gut caeca long, extending to the acetabulum. | <i>Opistholebes</i> |

Oesophagus very long and sinuous, many times length of the oral sucker. Gut caeca short, extending only to the middle of the body.

Gyliauchen

Subfamily 3. *Paracotylinae*, new. One testis only, behind ovary. Johnstone in 1911 described a parasite from the gills of the common dogfish, which he named *Paracotyle caniculae*. It resembles *Opistholebes amplicoelus* Nicoll, 1915, from which it differs in having one testis only. Type-genus: *Paracotyle* Johnstone, 1911.

FAMILY B. GASTROTHYLACIDAE STILES AND GOLDBERGER, 1910

Includes those paramphistomes which possess a ventral pouch. At present only 8 species are known, and these are included in 4 genera by Stiles and Goldberger (1910), 3 genera by Maplestone (1923), 1 genus and 3 subgenera by Fukui (1929) and 3 genera by Travassos (1934).

Fischöeder (1903) considered all paramphistomes with a ventral pouch as belonging to the one genus, *Gastrothylax* Poirier, 1883.

FAMILY C. CLADORCHIDAE STILES AND GOLDBERGER, 1910

Amphistomes in which the oral diverticula are paired in all species except *Stephanopharynx*. Ventral pouch absent. We recognize 7 subfamilies, which can be differentiated by the following key.

KEY TO SUBFAMILIES

- | | |
|--|----------------------------|
| Oral diverticulum single. | <i>Stephanopharynginae</i> |
| Oral diverticula double. | 1 |
| 1. { Posterior extremity divided into two :
(a) each part containing a vitelline gland ;
(b) each part without vitelline gland ; and collar
present round anterior extremity. | <i>Brumptinae</i> |
| { Posterior extremity not so divided. | <i>Kalitrematinae</i> |
| | 2 |
| 2. { Testes posterior to the ovary and uterus. | <i>Balanorchinae</i> |
| { Testes not posterior to the ovary and uterus. | 3 |
| 3. { Body divided into anterior and posterior parts,
the latter functioning as a large sucker. | <i>Gastrodiscinae</i> |
| { Body not so divided. | 4 |
| 4. { Acetabulum either divided into two parts, or
broader than body, or bearing an accessory sucker. | <i>Diplodiscinae</i> |
| { Acetabulum with none of the above characters. | <i>Cladorchinae</i> |

Subfamily 1. *Cladorchinae* Fischöder, 1901. With the characters of the family. Five genera can be differentiated by the following key :—

Genital sucker present.		<i>Cladorchis</i>
Genital sucker absent.		1
1. {	A lateral papilla on each side of acetabulum.	<i>Zygocotyle</i>
	Lateral papillae absent.	2
2. {	Papillae on ventral surface of body.	<i>Homalogaster</i>
	No papillae on ventral surface of body.	3
3. {	Pharynx present.	<i>Chiorchis</i>
	Pharynx absent.	<i>Pseudodiscus</i>

In view of the above limitations in the number of genera in this subfamily, we indicate below the genera which we place in synonymy.

Pseudodiscus Sonsino, 1895

SYNONYMS : *Pseudocladorchis* Daday, 1907.
Cleptodiscus Linton, 1910.
Pfenderius Stiles and Goldberger, 1910.
Wardius Barker and East, 1915.

Cladorchis Fischöder, 1901

SYNONYMS : *Stichorchis* Fischöder, 1901.
Taxorchis Fischöder, 1901.
Dadayius Fukui, 1929.
Chiostichorchis Artigas and Pacheco, 1932.

Chiorchis Fischöder, 1901

SYNONYMS : *Microrchis* Daday, 1907.
Hawkesius Stiles and Goldberger, 1910.
Schizamphistomum Looss, 1912.
Alassostoma Stunkard, 1917.
Schizamphistomoides Stunkard, 1925.
Tugumaea Fukui, 1926.
Ophioxenos Sumwalt, 1926.
Dadaytrema Travassos, 1931.
Travassosinia Vaz, 1932.
Nematophila Travassos, 1934.

Zygocotyle Stunkard, 1917. It is impossible to escape the conviction that *Stunkardia* Bhalerao, 1931, is identical with *Zygocotyle* Stunkard, 1917. We consider Bhalerao's species to belong to the genus *Zygocotyle*.

Subfamily 2. *Brumptinae* Stunkard, 1925. Includes all paramphistomes in which the posterior extremity is divided into two, each part containing a large vitelline gland. One genus only, viz., *Brumptia* Travassos, 1921.

Subfamily 3. *Kalitrematinae* Travassos, 1933. Paramphistomes in which the anterior extremity bears a collar, and the posterior extremity is divided into two by a U-shaped sulcus. One genus only, viz., *Kalitrema* Travassos, 1933.

Subfamily 4. *Balanorchinae* Stunkard, 1925. Paramphistomes in which the testes are posterior to the ovary and uterus. One genus only, viz., *Balanorchis* Fiscoeder, 1901.

Subfamily 5. *Gastrodiscinae* Monticelli, 1892. Paramphistomes in which the body is divided into an anterior and a posterior part, the latter functioning as a large sucker. With 2 genera, differentiated as follows :—

Papillae on the posterior disc-like portion.	<i>Gastrodiscus</i>
No papillae on the posterior disc-like portion.	<i>Gastrodiscoides</i>

The genus *Homalogaster* has hitherto been placed in this subfamily, the body being supposed to be divided into a large anterior portion, the ventral surface of which bears large papillae, and a small posterior part embracing the acetabulum. However, it appears to us that in *Homalogaster* the division of the body is not more pronounced than in certain other species of amphistomes not included in the *Gastrodiscinae*, such, for instance, as certain species in the *Diplodiscinae*. We have, therefore, thought it desirable to transfer this genus to the subfamily *Cladorchinae*.

Subfamily 6. *Diplodiscinae* Cohn, 1904. *Cladorchidae* in which the acetabulum is either divided into two parts or bears an accessory sucker. With 4 genera, differentiated as follows :—

Genital sucker present and anterior.	<i>Helastomatis</i>
Genital sucker absent.	1
1. { Acetabulum divided into two.	<i>Catadiscus</i>
{ Acetabulum not divided into two, but bears an accessory sucker.	2
2. { Pharynx present.	<i>Diplodiscus</i>
{ Pharynx absent.	<i>Opisthodiscus</i>

Along with other authors, notably Cort, Fuhrmann, Fukui and Hunter, we regard *Megalodiscus* as a synonym of *Diplodiscus*.

Subfamily 7. *Stephanopharynginae* Stiles and Goldberger, 1910.

Includes all paramphistomes which possess a single oral diverticulum. Ventral pouch absent. With 1 genus only, viz., *Stephanopharynx* Fiscoeder, 1901.

PRÉCIS OF CLASSIFICATION

In the above classification we recognize three families, separated by the presence or absence of oral diverticula, and by the presence or absence of a ventral pouch. The characters used for the differentiation of the subfamilies are

the number of testes and their position relative to the ovary; the divisions of the body; and the modifications of the acetabulum.

Amongst the characters utilized in the diagnosis of the various genera are the following :—

1. The position of the genital pore.
2. The proximity of the ovary to the testes and to the acetabulum.
3. The length of the oesophagus in terms of the diameter of the sucker.
4. The length of the gut caeca as compared with the length of the parasite.
5. The modifications of the acetabulum.
6. The presence or absence of a genital sucker, or pharynx, and of papillae on the body.

We have recognized 3 families, 11 subfamilies, and 26 genera. In all cases we have limited our conception of the amphistomes to those worms which possess a posterior acetabulum. Accordingly, we do not include the *Angiodictyidae* Looss, 1902, or other trematode parasites without an acetabulum, such as the genus *Megasolena* Manter, 1935, *Hapladena* Linton, 1910, and others.

FAMILY	SUBFAMILY
Superfamily PARAMPHISTOMOIDEA Stiles and Goldberger, 1910	PARAMPHISTOMINAE Fiscoeder, 1901
	OPISTHOLEBETINAE Fukui, 1929
	PARACOTYLINAE n.sf.
	PARAMPHISTOMIDAE FISCHOEDER, 1901
	GASTROTHYLACIDAE STILES AND GOLDBERGER, 1910
	GASTROTHYLACINAE Stiles and Goldberger, 1910
	CLADORCHIDAE STILES AND GOLDBERGER, 1910
	CLADORCHINAE Fiscoeder, 1901
	GASTRODISCINAE Monticelli, 1892
	DIPLODISCINAE Cohn, 1904
	STEPHANOPHARYNGINAE Stiles and Goldberger, 1910
	BRUMPTINAE Stunkard, 1925
	BALANORCHINAE Stunkard, 1925
	KALITREMATINAE Travassos, 1933

CLASSIFICATION

GENUS

SYNONYMS

—*Paramphistomum* Fiscoeder, 1901
 —*Cotylophoron* Stiles and Goldberger, 1910

—*Opistholebes* Nicoll, 1915
 —*Gyliauchen* Nicoll, 1915
 —*Telotrema* Ozaki, 1933
 —*Paragyliuchen* Yamaguti, 1934
 —*Cephaloporus* Yamaguti, 1934

—*Paracotyle* Johnstone, 1911

—*Taxorchis* Fiscoeder, 1901
 —*Stichorchis* Fiscoeder, 1901
 —*Dadayius* Fukui, 1929
 —*Chiostichorchis* Artigas and Pacheco, 1932

—*Gastrothylax* Poirier, 1883

—*Pfenderius* Stiles and Goldberger, 1910
 —*Cleptodiscus* Linton, 1910
 —*Wardius* Barker and East, 1915
 —*Pseudocladorchis* Daday, 1907

—*Cladorehis* Fiscoeder, 1901
 —*Homalogaster* Poirier, 1882
 —*Pseudodiscus* Sonsino, 1895
 —*Chiorehis* Fiscoeder, 1901
 —*Zygoecotyle* Stunkard, 1917

—*Microrchis* Daday, 1907
 —*Hawkesius* Stiles and Goldberger, 1910
 —*Shizamphistomum* Looss, 1912
 —*Alassostoma* Stunkard, 1917
 —*Shizamphistomoides* Stunkard, 1925
 —*Tugumaea* Fukui, 1926
 —*Ophioxenos* Sumwalt, 1926
 —*Dadaytrema* Travassos, 1931
 —*Travassosinia* Vaz, 1932
 —*Nematophila* Travassos, 1934

—*Gastrodiscus* Leuckart, 1877
 —*Gastrodiscoides* Leiper, 1913

—*Diplodiscus* Diesing, 1836
 —*Catadiscus* Cohn, 1904
 —*Opisthodiscus* Cohn, 1904
 —*Helastomatis* Fukui, 1929

—*Stunkardia* Bhalerao, 1931

—*Stephanopharynx* Fiscoeder, 1901

—*Brumptia* Travassos, 1921

—*Megalodiscus* Chandler, 1923

—*Balanorchis* Fiscoeder, 1901

—*Kalitrema* Travassos, 1933

KNOWN AMPHISTOMES FROM REPTILES, AFTER
TRAVASSOS, 1934 (EMENDED)

Host	Parasite
CHELONIDAE	
<i>Chelone mydas</i>	<i>Schizamphistoma scleroporum</i> (Creplin, 1844). <i>Schizamphistomoides spinulosum</i> (Looss, 1901). <i>Microscaphidium reticulare</i> (Van Beneden, 1859). <i>Polyangium linguatula</i> (Looss, 1899). <i>Polyangium miyajimai</i> Kobayashi, 1921. <i>Microscaphidium aberrans</i> Looss, 1902. <i>Angiodictyum parallelum</i> (Looss, 1902). <i>Octangium sagitta</i> (Looss, 1899). <i>Octangium hasta</i> Looss, 1902. <i>Octangium takanoi</i> Kobayashi, 1921. <i>Deuterobaris proteus</i> (Brandes, 1891). <i>Schizamphistoma scleroporum</i> (Creplin, 1844).
<i>Halichelis atra</i>	
TESTUDINIDAE	
<i>Pseudmys troosti</i>	<i>Alassostoma magnum</i> Stunkard, 1917.
<i>Pseudmys elegans</i>	<i>Alassostoma magnum</i> Stunkard, 1917.
<i>Pseudmys floridana</i>	<i>Alassostomoides parvum</i> (Stunkard, 1917).
<i>Pseudmys picta</i>	<i>Alassostomoides parvum</i> (Stunkard, 1917).
<i>Heosemys grandis</i>	<i>Chiorchis purvisi</i> sp. nov.
CHELYDRIDAE	
<i>Chelidra serpentina</i>	<i>Alassostoma parvum</i> (Stunkard, 1917).
DERMATEMYDAE	
<i>Dermatemys mawii</i>	<i>Schizamphistomoides tabascensis</i> Caballero and Sokoloff, 1934.
CHELIDIDAE	
<i>Chelis fimbriata</i>	<i>Nematophila grande</i> (Diesing, 1839).
<i>Hydraspis geoffroyiana</i>	<i>Nematophila grande</i> (Diesing, 1839).
<i>Hydraspis gibba</i>	<i>Nematophila grande</i> (Diesing, 1839).
<i>Hydraspis schopfii</i> ?	<i>Nematophila grande</i> (Diesing, 1839).
<i>Rhinemys nasuta</i>	<i>Nematophila grande</i> (Diesing, 1839).
PELOMEDUSIDAE	
<i>Podocnemis dumeriliana</i>	<i>Nematophila grande</i> (Diesing, 1839).
<i>Podocnemis expansa</i>	<i>Nematophila grande</i> (Diesing, 1839).
<i>Podocnemis tracaxa</i>	<i>Nematophila grande</i> (Diesing, 1839).
OPHIDIA	
<i>Thamnophis sirtalis</i>	<i>Ophioxenos denteros</i> (Sumwalt, 1926).
<i>Thamnophis ordinoides</i>	<i>Ophioxenos denteros</i> (Sumwalt, 1926).
<i>Herpethodrias fuscus</i>	<i>Catadiscus dolichocotyle</i> (Cohn, 1903).

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ON SOME PARASITIC WORMS FOUND IN *XENOPUS LAEVIS*, THE SOUTH AFRICAN CLAWED TOAD

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(Received for publication 13 April, 1937)

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ON A METACERCARIA FOUND IN THE PERICARDIAL SAC

CLASS TREMATODA

FAMILY STRIGEIDAE RAILLIET, 1919

SUBFAMILY ALARIINAE RAILLIET, 1919

Larval group *Diplostomulum* Brand., 1892

1. DESCRIPTION OF PARASITE

The larval trematodes here described, which are metacercariae, were found in the pericardial sac of *Xenopus laevis*. They were sent to us for identification by Dr. Slome, of the Royal College of Surgeons, London, along with a number of living toads. It is interesting to note that the frogs were shipped from South Africa to England about three years ago, and were kept in tanks containing ordinary tap-water, which was frequently changed. The fact that these metacercariae were noticed in the same species of frog at Capetown throws some light on the period of time during which they may survive. Presuming that the frogs were infected before coming to England (and the presumption is almost a certainty), then the length of life of these larval forms is considerable—three years at least. Bosma (1934) states that 'The longevity of the mesocercariae is unknown and therefore the possibility of their being carried over from one year

to the next is only supposed ; yet it seems entirely possible.' The author was also impressed with the remarkable tenacity of life exhibited by the mesocercariae even long after the death of their host. Every frog examined showed the presence of these larvae in the pericardial sac. They were alive and actively motile, and none was observed to be encysted. Striking changes in shape and size were noticed in the living worms (fig. 3) ; after death they exhibit a remarkable uniformity in size and appearance.

They are oval in shape, and measure about 500μ in length by 350μ in breadth, the greatest breadth being across the middle. In life they are capable of elongating to three times this length. At the anterior extremity, on each side of the oral sucker, the body is expanded ventro-laterally into a small tubercle or shoulder-like projection. The significance of these will be discussed later.

Viewed from either the ventral or the dorsal aspect, the metacercaria is perfectly flat and leaf-like. When turned on its side, however, it is apparent that the ventral surface is deeply concave or boat-shaped. This concavity is formed by the lateral margins being folded ventrally. These margins unite just short of the posterior extremity, thus dividing the larva into a hollowed-out anterior and a smaller cone-shaped posterior portion. The tubercles, noted before, are situated at the junctions of the anterior parts of the infolded lateral margins with the body. They represent the rudiments of the auricular projections, or tentacle-like appendages, which are found in some adult members of the subfamily *Alariinae*. In some species of the *Tetracotyle* group of larvae, such as *Tetracotyle iturbei* Faust, 1918, the sucking discs are quite apparent, but they develop in grooves and do not project outwards.

The cuticle shows well-marked transverse striations and has a uniform thickness of about 4.5μ . The entire cuticle is covered with minute spines between 1μ and 2μ in length ; these are largest and most abundant over the adhesive organ. On the anterior extremity, and particularly in the region of the oral sucker, the cuticle appears to bear a few very small papillae. The body contains numerous calcareous corpuscles (fig. 2), which are aggregated chiefly in the anterior three-quarters and are almost absent from the posterior quarter of the larvae. These corpuscles vary in diameter from 3μ to 11μ , and are present in such large numbers that they tend to obscure the internal structures.

The metacercariae are quite immature, and no trace of genital organs is apparent. In many species of strigeid metacercariae the rudiments of the genital organs are quite easily seen.

The mouth is ventro-terminal, circular, and leads into a well-defined oral sucker. This is triangular in shape, the base being formed by the mouth ; the apex points towards the posterior extremity. The length of the oral sucker is about 80μ , its greatest diameter 70μ , and the thickness of the muscular walls 13μ . The oral sucker leads directly into a muscular pharynx, which is an elongated oval in shape, and shows a well-marked pharyngeal cavity. The length of the pharynx is about 40μ , the breadth 30μ , and the thickness of the walls 10μ .

Posteriorly the pharynx opens into an exceedingly short oesophagus. In many specimens the two limbs of the gut diverge at the termination of the pharynx, no distinct oesophagus being visible. The greatest length is never more than 4μ . The gut caeca pursue a straight course, running parallel throughout almost their entire length, and embracing both the ventral sucker and the adhesive organ. At the posterior border of the latter organ they turn slightly towards the centre and then terminate. The posterior half of each caecum is broader than the anterior, and shows distinct lobing. The total length is about 250μ . The ventral sucker is oval in shape, measuring 70μ by 45μ , and its long axis lies transversely. It is situated on the mid-line, just posterior to its central point. The adhesive organ is subglobular in shape, and measures 100μ by 70μ ; it lies immediately posterior to the ventral sucker. Laterally, on either side, it is in direct contact with the gut caeca. The excretory bladder is V-shaped, and is situated in the cone-like posterior extremity. The two limbs diverge to run anteriorly and ventrally, each terminating close to the posterior ends of the gut caeca. A small channel joins each limb anteriorly; another channel was seen to run in the middle of the larva between the ventral sucker and the pharynx, but its junction with the excretory bladder could not be made out. We were unable to ascertain any further details relating to the reserve bladder system, either in living or in stained specimens.

We would call attention to the confusion which appears to exist with reference to the identity of the 'hold-fast' organ and the genital pore. Hunter (1928) figures the adhesive, or 'hold-fast,' organ as being immediately posterior to the ventral sucker in *Neascus wardi* Hunter, 1928. Hughes (1929) also figures the 'hold-fast' organ as being situated posterior to the ventral sucker in *Diplostomum scheuringi* Hughes, 1929. Faust (1918b), in a figure of *Tetracotyle iturbei* Faust, 1918, calls the 'hold-fast' organ the acetabulum, and what the preceding authors refer to as the ventral sucker (acetabulum) Faust names the primitive genital pore. Ward (1918) states that holostome larvae have the 'oral sucker rudimentary, much smaller than acetabulum. Genital atrium modified into sucking organ.' We follow the nomenclature of Hunter and Hughes.

2. CLASSIFICATION OF THE *Strigeidae*

La Rue (1926) placed in an order which he named *Strigeatoidea* those digenetic trematodes with cercariae which had forked tails. He recognized three suborders, *Strigeata*, *Schistosomata* and *Bucephalata*.

The suborder *Strigeata* embraced those forms with the following characters: body usually divided by a constriction into two parts, the anterior bearing the special organs of attachment, the posterior containing the major portion of the genitalia; the genital pore posterior; the acetabulum present, rudimentary or entirely lacking; a special 'hold-fast' organ on the ventral surface, posterior to the acetabulum; the cercariae with a forked tail, a pharynx and a true oral sucker.

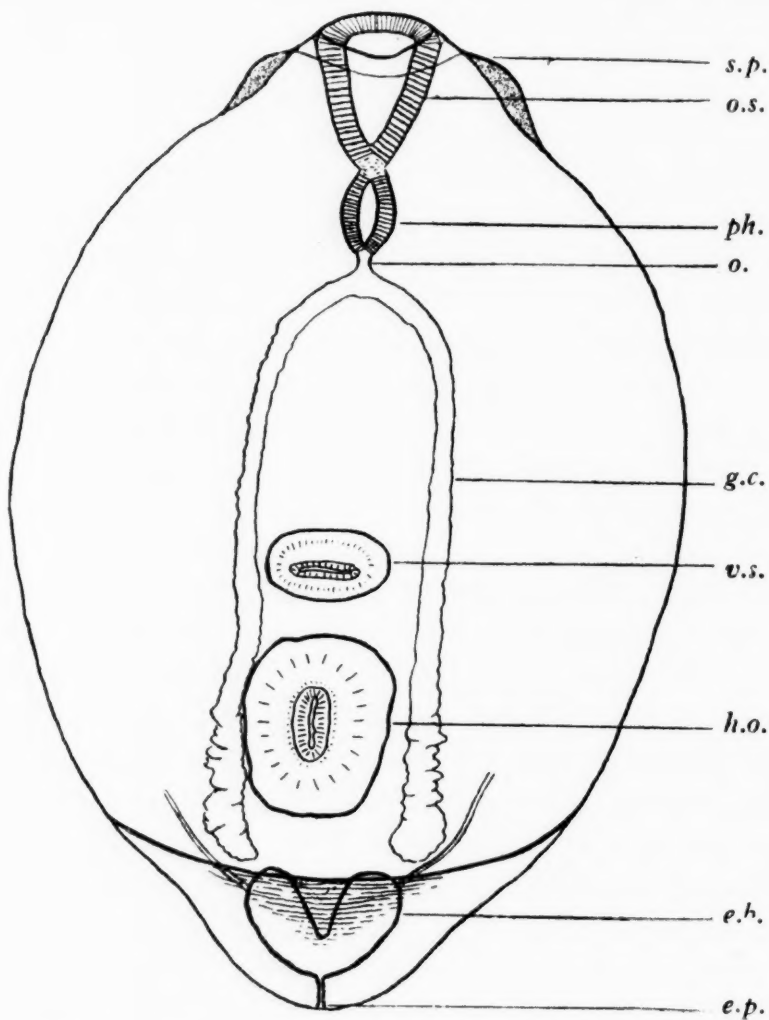


FIG. 1. Metacercaria, ventral view. (\times about 200.)

EXPLANATION OF LETTERING

e.h., excretory bladder.
e.p., excretory pore.
g.c., gut caeca.

h.o., hold-fast organ.
o., oesophagus.
o.s., oral sucker.

ph., pharynx.
s.p., shoulder-like processes.
v.s., ventral sucker.

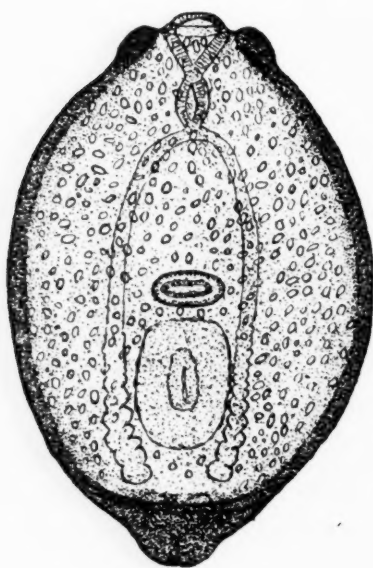


FIG. 2

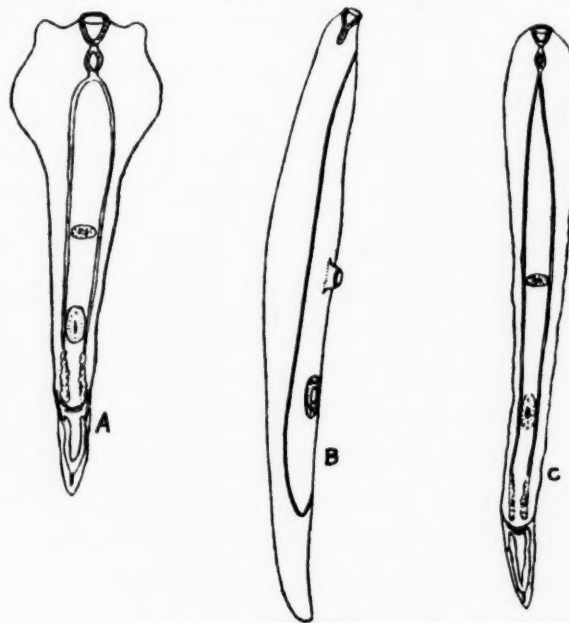


FIG 3

FIG. 2. Metacercaria, ventral view, showing calcareous corpuscles. (\times about 110.)
 FIG. 3. Metacercaria, showing changes in shape observed in living specimens.

The suborder *Strigeata* contained the type superfamily *Strigeoidea* Railliet, 1919, with the following characters: fore body region flattened, and incurved ventrally or cup-shaped; hind body region more or less cylindrical, ovoid or conical; intestinal caeca without diverticula, usually extending to the posterior extremity of the body; excretory systems forming a prominent net-work throughout the body; metacercariae in snails, leeches and vertebrates; adults in snails or fish-eating vertebrates.

FAMILY STRIGEIDAE RAILLIET, 1919.

Group *Holostomata* Lühe, 1909.

With the characters of the superfamily.

Subfamily 1. *Strigeinae* Railliet, 1919. Cirrus and cirrus pouch absent. Anterior part of the body cup-shaped. Organs of attachment always present.

Type-genus: *Strigea* Abildgaard, 1790.

Genus *Strigea* Abildgaard, 1790.

Synonym: *Holostomum* Nitzsch, 1819.

Anterior region sharply set off from posterior by a circular groove. Lateral margins curved ventrally to form a cup, with the mouth at the anterior extremity. Concealed in this cup is a small acetabulum and an adhesive organ in the form of a papilla extending to the mouth of the cup. The genital pore is situated on a well-developed genital cone; opening terminal.

Subfamily 2. *Alariinae* Hall and Wigdor, 1918. Cirrus and cirrus pouch absent. Anterior part of the body flat, usually having the lateral margins incurved ventrally; the posterior part is cylindrical and round or oval in cross-section. Lateral sucking cups (auricular projections or tentacle-like appendages) usually present near oral sucker. Organs of attachment greatly elongated or cordate.

Type-genus: *Alaria* Schrank, 1788.

Genus *Alaria*, Schrank, 1788.

Synonym: *Hemistomum* Diesing, 1850.

In the genus *Alaria* Schrank, 1788, the anterior region of the body is flattened and more or less expanded laterally, the lateral margins being incurved ventrally. The posterior region is cylindrical or flattened. On the auricular-like projections at the sides of the oral sucker there is usually a pair of accessory sucker-like organs, one on each side. In the absence of the latter, tentacle-like appendages may be present. A large 'adhesive organ' is present. There is no cirrus or cirrus sac. The vitelline glands are situated mainly in the anterior region of the body, and the genitalia in the posterior part.

From the above classification it is clearly evident that our larva, with its infolded lateral margins, cone-shaped posterior region, tentacle-like projections, and large 'hold-fast' organ, will develop into an *Alaria*.

3. LIFE-HISTORY OF CERTAIN SPECIES OF *Strigeidae*

Compared with a simple trematode life-history, like that of, say, *Fasciola hepatica* or *Schistosoma haematobium*, the life-histories of some species of this family appear to be very involved. In the briefest possible manner we indicate below the life-history of a few :—

1. *Strigea michiganensis* La Rue, 1927.

Synonym : *Cotylurus michiganensis* (La Rue, 1927) Haitzma, 1930.

Miracidium escapes from the egg and penetrates snail host ; develops into mother sporocyst, which give rises to innumerable secondary sporocysts ; these eventually produce bifid-tailed cercariae. Rediae stages do not occur. The bifid-tailed cercariae escape from the snail, enter fish, become metacercariae (*Tetracotyle*), and are found in the muscles and around the heart of various marine fishes. These fishes are eaten by herring-gulls, and the adult worms occur in the *bursa Fabricii*.

2. *Cotylurus flabelliformis* (Faust, 1917). Miracidium escapes from the egg and penetrates snail host ; develops into mother sporocyst, which gives rise to innumerable secondary sporocysts ; these eventually produce bifid-tailed cercariae. The latter escape into water, penetrate a second snail host, and in this host they develop into *Tetracotyles*. This second snail host is eaten by ducks, the *Tetracotyles* developing in the duck's intestine into adult worms.

3. *Diplostomum flexicaudum* (Cort and Brooks, 1928). Miracidium escapes from the egg and penetrates its snail host ; develops into mother sporocyst, giving rise to innumerable secondary sporocysts ; these eventually produce bifid-tailed cercariae. The latter penetrate the 'sucker' (*Catostomus commersonnii*) and travel to the eye of these fishes, eventually being found in the lens capsule, where they grow and metamorphose into the *Diplostomulum* stage. These fishes are eaten by herring-gulls, and the *Diplostomula* become adult worms in the intestine.

4. *Alaria mustelae* Bosma, 1931. Miracidium escapes from the egg and penetrates snail host ; develops into mother sporocyst, which gives rise to innumerable secondary sporocysts ; these eventually produce bifid-tailed cercariae. The latter penetrate a tadpole or frog, becoming immature metacercariae or agamodistomes. Bosma (1934) names these forms mesocercariae. The frogs or tadpoles are eaten by mammals such as mice, racoons and minks. In these mammals the mesocercariae develop into metacercariae, and in another mammal (mink or weasel) the metacercariae develop into adult worms.

The strange diversity in the life-history indicated above will serve to show the peculiar ways by which the adult stage is reached. Coming more particularly to those larval strigeids which have hitherto been recorded from amphibians, and from frogs in particular, we summarize below the results of the principal researches made in the past ten years or so.

4. TYPES OF STRIGEID METACERCARIAE

Lühe (1909) differentiates the following types of metacercariae within the family :—

1. Anterior end of body not funnel-shaped.

(a) Anteriorly no paired accessory sucking grooves.

(i) Body flat, leaf-like and broad. Posterior part passing into a cone corresponding to posterior part of adult worm.

Diplostomum
Nord., 1832

(ii) Body elongated, anterior end not broad and leaf-like.

Tylodelphys
Diesing, 1850

(b) On each side of the mouth (shoulders) one non-muscular sucking groove, in which lie the openings of the gland.

Tetracotyle
Filippi, 1854

2. Anterior end of the body elongated, cylindrical, shaped like a deep funnel.

Codonocephalus
Diesing, 1850

Faust (1918*b*) writes :—

'No end of confusion in the systematology of holostome larvae has resulted from a disregard for the original diagnosis of the genera together with ignorance of the life-history processes of the group. The genera *Diplostomulum*, *Tyrodelpys* [*sic*], and *Tetracotyle* have been recognized, but species of each of these have been placed in each of the other genera by overlooking items in the original description and by substituting incorrect descriptions for the genera to fit the cases in hand.'

Faust (1924) further states that :—

'A study of the larval characters of the trematodes for some years has brought me to the conclusion that there is only one common system carried over from the cercaria to the adult, which is sufficiently definite and conservative as to be utilizable for purposes of group identification. That system is the excretory system. The more work that is done in this system, the more indicative is it of possessing value as a natural basis of classification, and the more evident is the artificiality of some of Lühe's groupings of larval forms and of the equal artificiality of some of the families of adult trematodes that have been created. While the study of adult correlations with known larval forms is still in its infancy, it is not too much to state that all members of a natural adult group possess the same basic excretory pattern. There is no better exemplification of this than in the group of the human schistosomes where the excretory system in all three species of larvae is known to be identical and where the pattern in the adults is known to be a geometrical elaboration of this fundamental pattern in the larva. If, therefore, the correlation of one species of cercaria and adult trematode is known, it follows that other cercariae, having similar although specifically different larval characters, including the excretory or solenocyte pattern, belong to the same natural group.'

Hughes (1927) erected another larval group, which he named *Neascus*; it includes *Neascus ambloplitis* Hughes, 1927, and *Diplostomum cuticola* Nord., 1832. He defines the characters of his group *Neascus* as follows :—

'Strigeid metacercariae with both fore- and hind-bodies well developed and distinctly set apart by a constriction; no lateral sucking-cups; fore-body leaf-like; hold-fast organ

well-developed ; reserve bladder highly developed, the smaller branches of which are usually anastomoses ; calcareous granules mostly free in the circumambient fluid ; encysted.'

He compares his group *Neascus* with the other four groups thus :—

' *Neascus* differs from *Diplostomum volvens* von Nordmann as follows : (1) the absence of lateral sucking cups or ear-like processes ; (2) the hind-body is more extensively developed ; (3) the reserve bladder is more extensively developed and very different in character ; (4) the calcareous granules are not confined to vesicles at the termini of small branches ; (5) the smaller branches do not end blindly but constitute anastomoses, and (6) the forms are generally encysted.

' *Neascus* differs from *Tylodelphys* Diesing in that (1) a hind-body is present and (2) the calcareous granules are not confined to vesicles at the termini of small branches. . . .

' *Neascus* differs from *Tetracotyle* de Filippi as redescribed by Faust (1918 : 75) as follows : (1) the absence of lateral sucking cups, (2) the better development of the hind-body, (3) the shallowness of the concavity on the ventral side of the fore-body, which in *Tetracotyle* is usually deeply cup-shaped, and (4) the pattern of the reserve excretory bladder, which in *Neascus* is comprised of a system of definite tubules of various sizes, whereas in *Tetracotyle* there are only one or two large spaces interspersed with strands of parenchymal tissue.

' *Neascus* differs markedly also from *Codonocephalus* Diesing, the anatomy of which was worked out in detail by Kopczynski (1907 : 625). In *Codonocephalus* the fore-body is very much reduced in size, being present merely as a flattened disc at the anterior end of the hind-body, the latter being much more fully developed than it is in any of the other groups. The hold-fast organ is enclosed in a cavity communicating with the exterior through a very narrow tubular opening. The reproductive organs are very nearly mature. The excretory system constitutes a complicated network of vessels which Kopczynski (1907 : 642) divided into two groups, the central vessels and the superficial vessels.'

Van Cleave and Mueller (1934) discuss at some length the relationships of the larval groups *Diplostomulum*, *Tetracotyle* and *Neascus*. They conclude that the principal characters of these three groups are as follows :—

Diplostomulum. Nearly always found in the eyes of fishes ; never encysted ; body not clearly divided into two regions, although a small caudal lobe is usually present ; two lateral suckers or cotylae present ; oesophagus of varying length. (Reserve bladder system not discussed.)

Tetracotyle. Found encysted in the muscles and other organs ; body not definitely divided into two parts ; lateral cotylae present ; the reserve bladder system consisting of an irregular, continuous, coarse-meshed network of spaces not in the form of definite vessels.

Neascus. Encysted in viscera, skin and pericardium ; body divided into two ; with no lateral sucker-like organs on anterior end of body ; reserve bladder highly developed, smaller branches anastomosing.

5. REMARKS ON THESE LARVAE

It will be noted that Lühe's classification is based principally upon the shape of the body.

We have found it impossible to classify our species in any one of his four groups on the characters which he ascribes to them, and it is clear that this has been the experience of other investigators. Yet we would point out that

we had no difficulty in relegating our larva to the genus *Alaria*. We are of opinion that the groups *Diplostomum*, *Tylodelphys* and *Tetracotyle* are so similar that it is difficult, if not impossible, to distinguish between them. Clearly they all belong to the genus *Alaria*; it is equally clear that *Neascus* also belongs to the same genus.

The divisions of the body in the various groups, as defined by Hughes, does not appear to be recognized universally, and, as pointed out by Faust, difficulties have arisen in relegating a species to its proper group on the basis of the division of the body into two. It is for this reason that we think it advisable to group them all together under the one name *Diplostomulum*. It is significant that in recent years very few observers have attempted to classify their larvae in any of these groups, but have contented themselves with referring to them as metacercariae.

The group *Codonocephalus* apparently contains a single species only, namely, *Codonocephalus urnigerus* (Rud., 1819) = *C. mutabilis* (Diesing, 1850). It is obvious that this larval form belongs to the genus *Strigea*.

Cobbold (1861), with reference to the two groups which we have retained, wrote in 1859 as follows:—

‘ DIPLOSTOMA (*Diplostomum*), Nordm.’

‘ Were it possible even with only a moderate degree of accuracy to refer to their true adult representatives the several individuals here associated together, I should be glad to cast aside this genus entirely; but, while accepting in a general sense the view of those who maintain that the Diplostomes are merely larval or immature Holostomes, how, I ask, are we to allocate under their proper specific titles even those forms which have been most frequently subjected to investigation? The differing views of Steenstrup, Siebold, Aubert, Wagener, Claparède, and others, are at present irreconcilable, and the difficulty is still further enhanced by the surprising results of Leuckart’s researches among the Pentastomata, where he finds that even the presence of well-developed reproductive organs affords no safe criterion of the adult state, for, in these crustaceoid parasites they exist in the pupa condition. As this obtains in the Pentastomes, may not the evidence equally apply to the digenetic flukes? Without at present entering into the discussion, I think it advisable, if only for the sake of convenience, to retain this genus until some one shall have more conclusively established the affinities and alternating relations in the cycle of development, as they affect, at least, a proportion of the so-called species.’

‘ CODONOCEPHALUS. (*Diesing.*) ’

‘ In the absence of any original examination of this Trematode, I am unwilling to set aside Diesing’s generic distinctions, but I find Wedl who has recently contributed several careful papers on Entozoology, remarking in his “Anatomische Beobachtungen über Trematoden,” that, in his opinion, this genus can scarcely be legitimately maintained. In a group of such numerous and closely-allied forms as the Trematodes present, one is almost necessitated to employ as generic, characters which have only a subgeneric value; yet, at the same time, it is essential to avoid as far as possible the splitting up of groups.’

The erection of a new group, namely, *Neascus*, by Hughes in 1927 initiates a new character on which it is suggested that the future classification of these metacercariae should be based. In this group the important organ is the so-called ‘reserve bladder’ and its principal vessels. As far as we are aware, the ‘reserve bladder’ system is limited to the *Strigeidae*.

It is admitted that the primary excretory apparatus is of the utmost importance in the classification of cercariae. In strigeid metacercariae the primary excretory apparatus is superseded and entirely eclipsed by the development of a very prominent secondary excretory ('reserve bladder') system.

The 'reserve bladder' system can only be seen properly in living specimens, but we believe that to determine the distribution of even the principal vessels is a matter of considerable difficulty. In our specimens we were unable to see any trace of the 'reserve bladder,' except what appeared to be a rather large vessel running antero-posteriorly in the mid-line of the parasite.

The difficulty in tracing vessels, their branches and connections, together with the fact that the system can only be determined in living animals, constitutes, in our opinion, a serious practical difficulty, more especially on account of the fact that the material is usually received by the investigator in a preserved condition. The transition from the primary to the secondary system is, we know, sudden and dependent on the larva reaching another host. We are aware that the excretory system in mesocercariae is but a relatively slight elaboration of the primary system found in cercariae; the 'reserve bladder' does not begin to develop until the larva reaches its next host, which is sometimes a mammal.

Our experience in the classification of various groups of animals has proved to us repeatedly that the variations which may, and do, occur in any organ or system are often extreme, and we have every reason to believe that similar variations will be found to occur in the secondary excretory system. Nevertheless, we can readily understand that the determination of the 'reserve bladder' in any metacercariae might be extremely useful as indicating the natural group to which the adult worm might belong; but, as this secondary excretory system has at present little or no value as a diagnostic character in the adult, its utility is limited, principally, to the metacercarial stage. We have already said that its usefulness in the identification of cercariae is undoubted, but in these cases the system is infinitely more simple.

6. COMPARISON OF OUR LARVA WITH OTHER STRIGEID LARVAE RECORDED FROM AMPHIBIA

1. *Tetracotyle crystallina* (Rudolphi, 1819). In this species there is a large genital pore (? acetabulum); the lateral suckers bear small spines; the excretory bladder is rhomboidal, and the larvae are encysted in the muscles. We do not think that our species is *Tetracotyle crystallina*, because the latter has no calcareous corpuscles, and also the larvae are found encysted. Apparently the spines in this species are limited to the lateral suckers, whereas in our species they cover almost the entire body.

2. *Codonocephalus urnigerus* (Rudolphi, 1819).

Synonym: *Codonocephalus mutabilis* (Diesing, 1850).

This species is found encysted in the subperitoneal tissues of *Rana esculenta*.

The cysts measure from 2 mm. to 3 mm. in diameter. The characters of this parasite are so striking (see page 251) that a comparison is unnecessary. Calcareous corpuscles are present in this larval form.

3. *Tetracotyle pipientis* Faust, 1918. In this species the body is densely covered with spines, and a crown of denser and bigger spines surrounds the genital pore (? acetabulum); the acetabulum is modified into a single transverse lappet. The pharynx is small, the excretory bladder inconspicuous, and calcareous corpuscles are absent. In our species the pharynx is large and the excretory bladder prominent; calcareous corpuscles are present. Incidentally, *Tetracotyle pipientis* is encysted in a stout capsule in the mesentery and peritoneum, whilst our species is found free in the pericardial fluid.

4. *Diplostomulum joyeuxi* Joyeux and Baer, 1934. The metacercariae of this species have been recorded by Joyeux and Baer encysted in the muscles of *Rana esculenta*. They measure from 700μ to 1 mm. in length, by 450μ to 550μ in breadth. They are thus very much larger than our larvae. In this particular, and in the absence of calcareous corpuscles, they differ from our species.

5. *Diplostomulum tetracystis* (Gastaldi, 1854). This larval form was found encysted in a gelatinous mass in the thorax of *Rana catesbiana*. The capsules measure 800μ by 700μ , and the worms are able to free themselves rapidly from their delicate connective tissue capsules. The immature parasites measure from 700μ to 1 mm. in length. The body is covered with spines. There is a very long oesophagus, and the intestinal caeca are short. This larval form, according to Bosma (1934), is a *Diplostomulum* (= *Diplostomum*). No mention is made of a 'hold-fast' organ, and in any case the species is quite different from ours in that it possesses a long oesophagus and short intestinal caeca.

6. *Mesocercaria mustelae* Bosma, 1934.

Adult: *Alaria mustelae* Bosma, 1931.

The mesocercariae ('agamodistomes') occur in tadpoles and frogs. The larval form measures from 240μ to 340μ in length, and from 143μ to 205μ in breadth. It is elongated, oval and flattened dorso-ventrally. There may be a slight indication of a hind body. The cuticle is covered with spines; the pharynx is small, the oesophagus and pre-pharynx short, and the intestinal caeca extend posteriorly four-fifths the length of the worm. This form differs from our species in possessing genitalia, and in the scarcity of calcareous corpuscles. Further, only the mesocercariae occur in frogs, and these are found in the lymph spaces, between the muscle fibres, and on the wall of the alimentary canal. Our specimens are metacercariae, and occur in the pericardial sac.

7. *Mesocercaria marciana* Bosma, 1934.

Synonyms: *Cercaria marciana* La Rue, 1917.

Agamodistomum marciana Cort, 1918.

This species has been recorded from the tissues and lymph spaces of *Rana pipiens* and *Rana clamitans*. It is a mesocercaria in which the 'hold-fast' organ has not developed, and we cannot, therefore, compare it with our metacercaria. The body is covered with spines; the acetabulum is large and appears to be placed more anteriorly than is the case in our species.

These mesocercariae were fed to snakes, and did not develop into metacercariae, but migrated from the gut into the body cavity and tissues of the snake.

8. *Diplostomum* sp. Ercolani (? 1855). Braun (1879-93, p. 870) records this larval form from *Rana temporaria*. He also records *Distomum* sp. Perroncito (? 1880), a larval form encysted in *Rana esculenta*. Neither of the above species is mentioned in the Index-Catalogue (Trematodes) (Stiles and Hassall, 1908), and we have been unable to obtain any information regarding them.

9. *Distomum pelophylacis esculenti* Wedl, 1849.

Synonym: *Distomum wedlii* Cobbold, 1860.

This parasite was found encysted in the brain of *Rana esculenta*. We have been unable to obtain any information regarding it, but are of opinion that, as it is encysted and occurs in the brain, it seems hardly likely to be identical with ours.

10. *Tylodelphys rhachiaea* (Henle, 1833). Lühe (1909) states that this form measures 500μ by 250μ , but is capable of great variations in shape. The pharynx is prominent, and there is no oesophagus; the gut caeca extend to the posterior margin of the 'hold-fast' organ. The genital pore (acetabulum) is prominent, and is situated immediately in front of the 'hold-fast' organ. The body of the parasite contains numerous calcareous corpuscles. The worm is found in the cerebrospinal canal of frogs, especially round the cauda equina, frequently in large numbers.

In both *Tylodelphys rhachiaea* and our species the acetabulum and 'hold-fast' organ are situated behind the middle of the parasite. It is not stated whether the lateral margins of this species are curved ventrally, but Lühe figures a perfectly flat larva. In our species, as noted previously, the lateral margins are infolded, a fact which can be ascertained only by examining the larva 'in profile.' Furthermore, no mention is made by Lühe of minute spines. Nevertheless, there is a striking resemblance between the two parasites, and we have no option but to conclude that our form belongs to this species. The adult form of *Tylodelphys rhachiaea* is not known; Lühe (1909) suggests that it is *Hemistomum excavatum* (Rud., 1803), but this appears improbable.

Since our species is a metacercaria, we can only presume that the larva develops into an adult in some animal, such as a snake or a bird, which feeds on frogs.

ON A NEW SPECIES OF NEMATODE, *PROCAMALLANUS SLOMEI*
SP. NOV.

SUPERFAMILY **Spiruroidea** RAILLIET AND HENRY, 1915

FAMILY CAMALLANIDAE RAILLIET AND HENRY, 1915

Genus (1) *Procamallanus* Railliet and Henry, 1915

Procamallanus slomei sp. nov.

Six males and 12 females were obtained from the stomach, the heads being deeply embedded in the wall. They were easy to recognize, on account of their red colour, due to the presence of haemoglobin in the intestine and body cavity. At least one worm was found in every individual of a batch of 10 toads.

The males vary in length from 1.8 mm. to 2 mm., and in breadth from 0.09 mm. to 0.15 mm. The cuticle is more coarsely striated in the male than in the female. The excretory pore is situated in the region of the nerve ring. The tail of the male is coiled ventrally and is bluntly rounded; it has an average length of 0.06 mm. Anal alae are present and conspicuous. There are usually 12 pairs of papillae on the tail: 8 pairs preanal, 1 pair adanal and 3 pairs postanal, all of which are pedunculated. The mouth bears 6 papillae—2 lateral and 4 median. The brownish-yellow buccal capsule is broadly barrel-shaped, and its anterior opening is hexagonal. It varies in length from 0.087 mm. to 0.097 mm., and in breadth from 0.065 mm. to 0.075 mm. The internal surface of the buccal capsule is perfectly smooth. The oesophagus is divided into the usual two parts. The anterior muscular part is club-shaped and measures from 0.2 mm. to 0.24 mm. in length. The posterior glandular and somewhat longer part measures from 0.22 mm. to 0.29 mm. in length, and, unlike the muscular part, has a uniform breadth. The intestine was conspicuous on account of its being full of partly digested blood. The nerve ring encircles the muscular oesophagus at the junction of the anterior and middle thirds. The testis is a long, thin, convoluted tube situated in the region of the glandular oesophagus. It opens rather abruptly into a larger elongated vas deferens, of which the anterior extremity is dilated into a seminal vesicle. Posteriorly, the vas deferens shows a marked constriction where it passes into the ductus ejaculatorius. Part of the latter organ is surrounded by a cement gland. Only one spicule was noticed: it measured from 0.087 mm. to 0.1 mm. in length.

The females vary in length from 2.5 mm. to 2.9 mm., and have a maximum diameter of about 0.22 mm. The posterior extremity is bluntly rounded and terminates in a crown of spines varying from 8 to 10 in number. The vulva is situated just anterior to the middle of the worm. The buccal capsule averages 0.135 mm. in length and 0.097 mm. in breadth; the muscular oesophagus measures about 0.26 mm., and the glandular oesophagus about 0.29 mm. in length. The ovary arises in the region of the muscular oesophagus and passes into the oviduct, which then enters the large sac-like double uterus. The latter extends from the muscular oesophagus almost to the posterior extremity; its walls are very corrugated and in life show marked powers of contractility.

The ovejector arises at the junction of the two uteri ; it is a long tube which passes to the vulva and terminates in a very short brown-walled vagina. In every specimen the uterus contained active larvae. These measured up to 0.25 mm. in length and 0.014 mm. in breadth, and had long pointed tails. Eggs were also seen in the uteri, some of which contained a segmenting ovum, whilst others contained a larva.

DIAGNOSIS

As far as we are aware, the only species of the genus *Procamallanus* which has been recorded from batrachians is *P. xenopodis* Baylis, 1929, obtained from the toad *Xenopus mülleri*. The material consisted of 6 worms, 3 young males and 3 very immature females. Allowing for this immaturity, the measurements of *P. xenopodis* are very similar to those of the above species. The main points of difference are in the buccal capsule and in the crown of spines on the female tail. The buccal capsule of *P. xenopodis* is marked with interrupted and irregular ridges tending to take a diagonal or spiral direction, whereas in our species the buccal capsule is perfectly smooth. In *P. xenopodis* the tail of the female appears to have a constant crown of 7 spines ; in our species there are from 8 to 10 spines, usually 10. Baylis (1929) states that 'The spicules are unequal, the left being apparently somewhat shorter than the right and much more slender.' He did not, however, give the measurements of the left spicule. As noted above, we were not able to demonstrate the left spicule.

Up to the present, 18 species of *Procamallanus* have been recorded, all of them, except *P. xenopodis*, from fishes :—

- P. laeviconchus* (Wedl, 1862).
- P. spiralis* Baylis, 1923.
- P. parasiluri* Fujita, 1927 (females only).
- P. iheringi* Travassos, 1928.
- P. inopinatus* Travassos, 1928.
- P. rarus* Travassos, 1928.
- P. xenopodis* Baylis, 1929.
- P. mehrii* Agarwal, 1930.
- P. sphaeroconchus* Törnquist, 1931.
- P. kerri* Pearse, 1933 (single female only).
- P. amarali* Vaz and Pereira, 1934.
- P. hilarii* Vaz and Pereira, 1934.
- P. planoratus* Kulkarni, 1935.
- P. wrightii* Pereira, 1935.
- P. barroslimai* Pereira, 1935.
- P. fariasi* Pereira, 1935.
- P. sigani* Yamaguti, 1935.
- P. fulvidraconis* Li, 1935.

Of all the above species only three possess a smooth buccal capsule, viz.,

P. laeviconchus, *P. planoratus* and *P. sigani*. They differ from our species in the following points :—

	<i>P. slomei</i> sp. nov.	<i>P. planoratus</i>	<i>P. laeviconchus</i>	<i>P. sigani</i>
Spicules	Right, 0.11 mm. Left, —	Equal, 0.12 mm.	Right, 0.15 mm. Left, 0.05 mm.	Right, 0.4 mm. Left, 0.12–0.15 mm.
Size	Male, 1.8–2 mm. by 0.12 mm. Female, 2.5–2.9 mm. by 0.22 mm.	3.85 mm. by 0.061 mm. 5.07–7.26 mm. by 0.079 mm.	3.65 mm. by 0.11 mm. Up to 15.5 mm. by 0.35 mm.	10 mm.–14 mm. 19 mm.–27 mm.
Female tail	Blunt. Crown of 8–10 spines	Blunt. No crown	Conical, tapering. Three very short blunt processes	No crown
Male papillae	8 pairs preanal 1 pair adanal 3 pairs postanal	7 pairs preanal 1 pair postanal	9 pairs preanal 1 pair adanal 4 pairs postanal	3 pairs preanal 6 pairs postanal

Pearse (1933) found four spiruroids in the intestine of *Glossogobius giurus*. He erected a new genus, *Thelazo*, in the family *Thelaziidae* to contain these worms. The characters which he gave to the genus are as follows : 'Buccal capsule is a chitinous, barrel-shaped cavity, without valves or lips. The posterior end of the male is coiled ; gubernaculum present ; spicules unequal ; no caudal alae. Vulva near anterior end.'

The buccal capsule, as figured by Pearse, is typically that of *Procamallanus*, and, from the description and figures given, this parasite must be included in that genus. It resembles our species in having a smooth buccal capsule, but differs from it in the following important points :—

	<i>Procamallanus slomei</i> sp. nov.	<i>Thelazo glossogobii</i>
Male caudal alae	Present	Absent
Male papillae	13 pairs	6 pairs
Female tail	Crown of ten spines	Three short divergent spines

We have pleasure in naming this parasite in honour of Dr. Slome, who very kindly sent us the toads for examination.

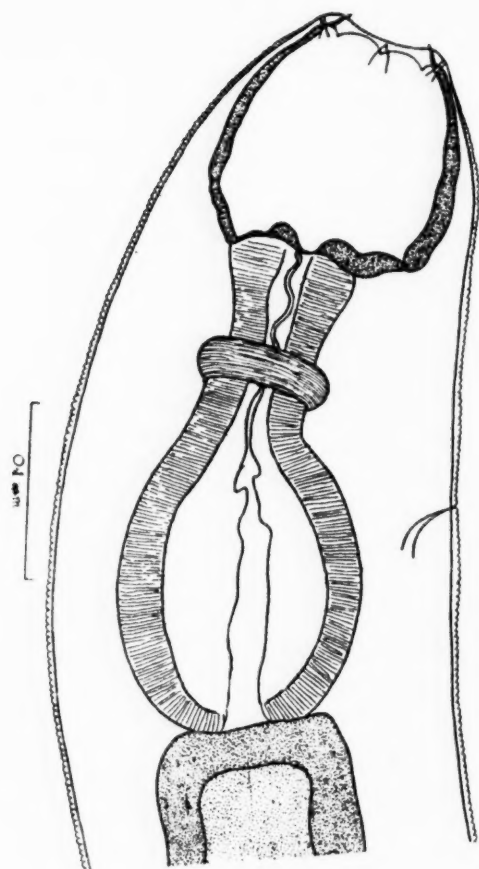


FIG. 4

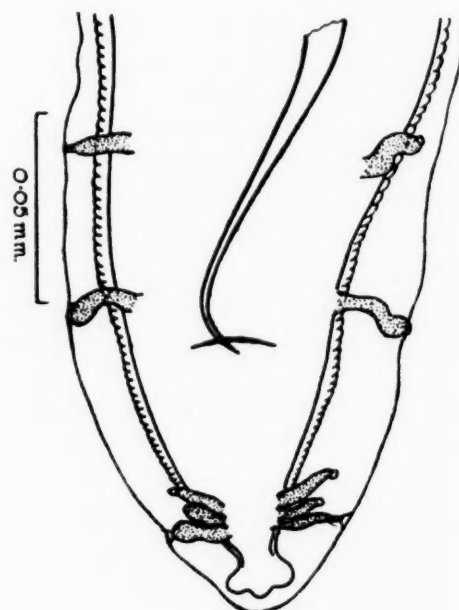


FIG. 6

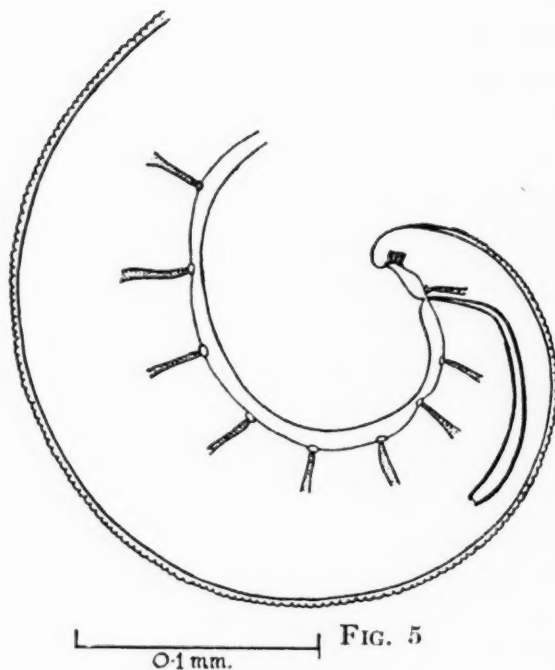


FIG. 5

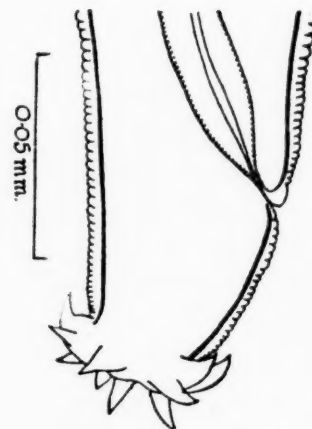


FIG. 7

- FIG. 4. *Procamallanus slomei* sp. nov. Head, lateral view.
 FIG. 5. *Procamallanus slomei* sp. nov. Tail of male, showing papillae and spicule, lateral view.
 FIG. 6. *Procamallanus slomei* sp. nov. Tail of male, ventral view.
 FIG. 7. *Procamallanus slomei* sp. nov. Tail of female, showing crown of spines.

ON A NEW SPECIES OF NEMATODE, *CAMALLANUS KAAPSTAADI*
SP. NOV.

Genus (2) *Camallanus* Railliet and Henry, 1915.

Camallanus kaapstaadi sp. nov.

Five males and 7 females of this species were also obtained from the stomach. As in the case of *P. slomei* sp. nov., the heads were deeply embedded in the stomach wall, and the worms were easy to recognize on account of their red colour.

The males vary in length from 2.5 mm. to 3 mm., and in breadth from 0.17 mm. to 0.2 mm. Cuticular striations are present. The excretory pore is situated in the region of the nerve ring. The tail has an average length of 0.09 mm., and terminates in two short spines. Anal alae are present and conspicuous. There are 12 pairs of papillae on the tail: 6 pairs preanal, 1 pair adanal, and 5 pairs postanal. The mouth bears 6 papillae, 2 lateral and 4 median. The buccal capsule consists of the usual two lateral plates and tridents. The internal surface of each valve is marked by linear cuticular ridges running antero-posteriorly. These vary in number from 30 to 36. The capsule is brownish-yellow in colour, definitely broader than long, and measures on the average 0.1 mm. in length and 0.12 in breadth. Its base is supported by a chitinous ring which marks the junction of the capsule with the oesophagus. The latter is divided into two parts, viz., an anterior club-shaped part, measuring about 0.28 mm. in length, and a posterior glandular part, which has a length of about 0.36 mm. and a breadth of 0.07 mm. The male genitalia resemble those of *P. slomei* sp. nov. The right spicule is sheathed and averages 0.33 mm. in length, and the left 0.2 mm. in length.

The females measure up to 4.8 mm. in length and 0.45 mm. in breadth. The posterior extremity terminates in three pointed spines. The vulva is situated well behind the middle of the worm, about three-eighths the length of the worm, from the tip of the tail. The buccal capsule measures up to 0.12 mm. in length and 0.16 mm. in breadth.

The club-shaped muscular oesophagus measures up to 0.35 mm. in length, and the glandular part up to 0.5 mm. in length. The female genitalia are like those of *P. slomei* sp. nov. The uterus contained active larvae in every specimen. These measured about 0.29 mm. in length and possessed long pointed tails.

DIAGNOSIS

According to Walton (1935) 5 species of this genus have been recorded from amphibians:—

Camallanus microcephalus (Dujardin, 1845) Railliet and Henry, 1915.

Camallanus nigrescens (v. Linstow, 1906) Railliet and Henry, 1915.

Camallanus baylisi Karve, 1930.

Camallanus multiruga Walton, 1932.

Camallanus pipientis Walton, 1935.

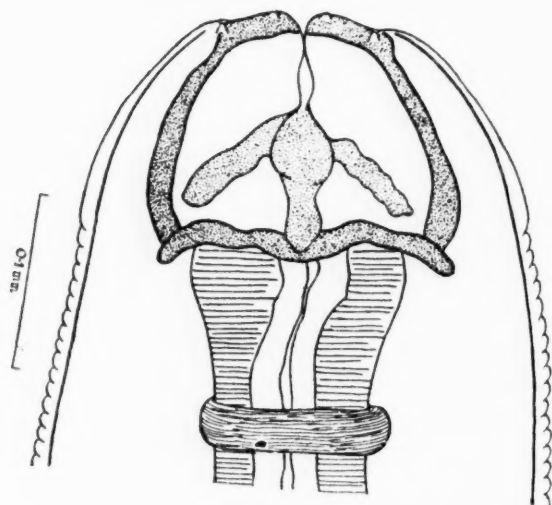


FIG. 8

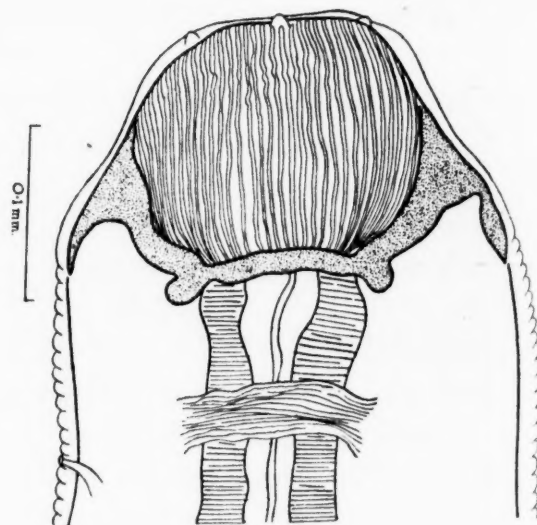


FIG. 9

FIG. 8. *Camallanus kaapstaadi* sp. nov. Head, ventral view, showing trident.
 FIG. 9. *Camallanus kaapstaadi* sp. nov. Head, lateral view.

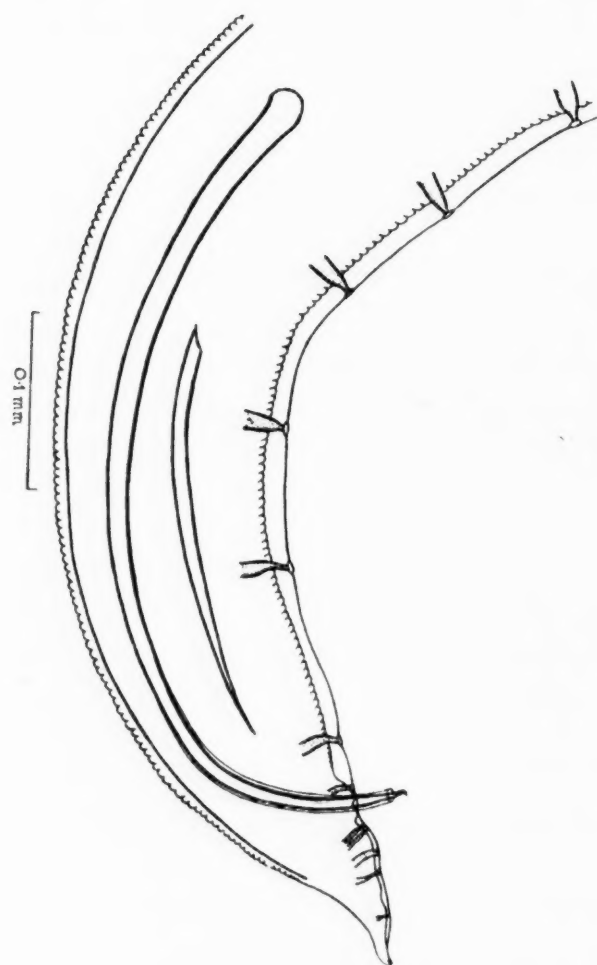


FIG. 10

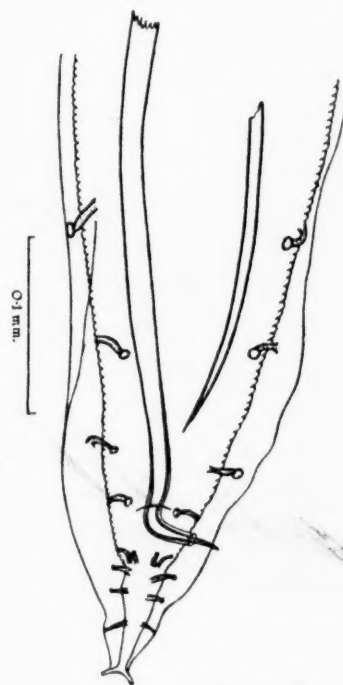


FIG. 11

FIG. 10. *Camallanus kaapstaadi* sp. nov. Tail of male, lateral view.
 FIG. 11. *Camallanus kaapstaadi* sp. nov. Tail of male, ventral view.

The above have been found in species of *Rana*, except possibly *C. multiruga*, the host of which is merely listed as a 'frog.'

Walton gives a table showing the characters of the five species in question. The points of difference between our parasite and these five are :—

1. Its much smaller size, even though the specimens are fully mature.
2. In no other species is the tail of the male bifid and that of the female trifid.
3. The only other species the male of which has the same number of anal papillae as ours is *C. microcephalus*, but this parasite is almost three times the size of our species. Furthermore, the tail of the male is not bifid, and the distribution of the papillae is different. The host of *C. microcephalus* is *Rana pipiens* from Wisconsin, U.S.A.

We therefore consider our parasite to be a new species, and we accordingly name it *Camallanus kaapstaadi*, the latter being the Dutch word for Capetown.

A NOTE ON THE OCCURRENCE OF *CEPHALOCHLAMYS NAMAQUENSIS* (COHN, 1906)

CLASS CESTODA

FAMILY DIPHYLLOBOTHRIIDAE LÜHE, 1910

SUBFAMILY DIPHYLLOBOTHRIINAE LÜHE, 1910

Genus *Cephalochlamys* Blanchard, 1908

Cephalochlamys namaquensis (Cohn, 1906).

Synonyms : *Chlamydocephalus namaquensis* Cohn, 1906.

Dibothriocephalus xenopi Ortlepp, 1926.

This parasite was found in the intestine of nearly all the toads. Adequate descriptions of the worm are given by Cohn and Ortlepp. We would remark, however, that the eggs are not operculated, but there is a micropyle at one extremity. They contain a hooked embryo.

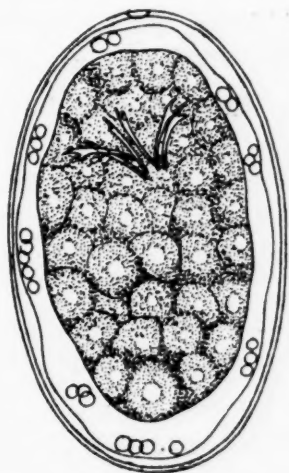


FIG. 12. Egg of *Cephalochlamys namaquensis*

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INFECTIVITY OF THE SALIVA IN PARALYTIC RABIES

BY

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(Received for publication 8 March, 1937)

Excessive salivation is a common feature of paralytic rabies in man and animals in Trinidad. Among bovines the condition is often so marked that, in spite of repeated advice and admonition, peasant owners and stock-tenders scoop out with their bare hands masses of watery or frothy mucus from the mouths of affected animals. No person has, however, so far been found to have contracted the disease through this manipulative procedure.

There is some difference of opinion as to the dangers attendant upon contact of the infected saliva of rabid animals with the abraded skin in the absence of the entrance of infection into the deeper tissues. Babes and Vasilu (1911), for instance, failed to cause infection with street virus rubbed into superficially produced scratches; on the other hand, there are several authentic records of the development of the disease through the licking of the hands by infected animals. With regard to the saliva of rabid human beings, Kraus, Gerlach and Schweinburg (1926) have pointed out the discordance in the results and opinions concerning its infectivity, adding that no case of rabies has been known to be produced by the bite of a human being. Joseph Koch (1930) reported cases of individuals who had been bitten by persons suffering from rabies, but who failed to develop the disease. He further noted that only two cases are on record of the direct transmission of the disease from one human being to another: in one instance infection took place during coitus, and in the other an atypical form of paralytic rabies developed after a bite. Palawandow and Serebrennaja (1930), however, produced typical rabies in guinea-pigs, with the formation of Negri bodies, by the intramuscular injection of saliva from a woman suffering from rabies, and in 1931 obtained similar results from five rabid persons. In view of the widespread nature of the disease among animals, and the close association of the peasantry with their livestock, it became a matter of interest and importance to determine experimentally the degree of infectivity of the saliva in cases of the disease as it occurs locally.

I. SALIVA FROM ANIMALS

The saliva was removed with sterile swabs from the mouths of 6 bovines and 2 horses between the 3rd and 5th day of the disease, and was immediately

rubbed on to the superficially scarified abdominal wall of rabbits S1 to S8. Paralysis of one or other limb took place, followed by death, as below :—

TABLE I

Rabbit no.	No. of days after infection in which paralysis developed	No. of days after infection in which death took place	Negri bodies
S1	9	11	+
2	12	12	+
3	8	9	+
4	9	9	?
5	6	8	—
6	15	15	+
7 (horse)	8	8	—
8 (horse)	7	9	+

Further inoculations were made intra-ocularly into 2 other rabbits from the brains of S4 and S5. Death took place with paralysis in 10 and 12 days respectively, but no Negri bodies could be seen.

II. SALIVA FROM HUMAN BEINGS

By a similar method, between the 2nd and 4th day of illness, the saliva from 6 persons with the symptoms of paralytic rabies was rubbed into the scarified abdominal wall of 7 rabbits, with the results shown in Table II.

TABLE II

Patient	Rabbit	No. of days after infection in which paralysis developed	No. of days after infection in which death took place	Negri bodies
P.O.	RS 1	9	10	+
C.B.	" 2	18	18	+
"	" 3	70	71	+
V.M.	" 4	3	4	—
J.D.	" 5	16	19	+
E.D.	" 6	10	10	+
C.C.	" 7	19	21	+

The brains of rabbits 1, 4 and 5 were further inoculated into the right sciatic nerve of 3 other rabbits. Death took place in 12, 18 and 18 days

respectively, and Negri bodies were present in their hippocampus. Patients V.M. and E.D. were remarkable in the fact that there was very little salivation, and the swabs, though rubbed over the tongue, were just moist. Infection nevertheless took place readily.



Paralytic rabies with salivation

III. SALIVA FROM VAMPIRE BATS

Previous experiments have shown that paralytic rabies is spread in man and animals by the bite of the vampire bat. Pawan (1936) also showed that the direct inoculation with a sterile swab of the saliva from 5 infected bats on to rabbits also produced the disease, death taking place in 19, 14, 11, 7 and 28 days. In the 4th instance, where death took place in 7 days, a dry stick was merely moistened with the saliva from the tongue of the infected bat and rubbed into the scarified abdomen of the rabbit. Except in one case, Negri bodies were present in the hippocampus of the infected rabbit. It should here be mentioned that the scarification of the back of a *Macacus rhesus* monkey with the saliva of an infected bat failed to produce infection, though previous work had shown these monkeys to be susceptible to rabies. Maybe the disproportion between body weight and quantum of virus accounted for the resistance to infection.

Manouélian (1935) attributes the high infectivity of the saliva to the escape of the virus from the minute nerve-cells of the tongue and the mucous membrane of the mouth; in these, he has demonstrated the presence of Negri bodies and of parasites which, he claims, belong to the group of 'formations' of which Negri bodies constitute a variety.

SUMMARY

1. The saliva of bovines, horses, bats and human beings suffering from paralytic rabies is highly infectious to rabbits.

2. Though a large number of persons scoop out the saliva from the mouths of rabid animals with their hands, upon which superficial wounds can be seen, no one has, so far, contracted the disease through this practice.

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A CASE OF SICKLE-CELL ANAEMIA IN TRINIDAD

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Since Herrick (1910) first reported on the condition as a distinct clinical entity in Panama, cases of sickle-cell anaemia have been recorded with increasing frequency in the United States. It was thought that the affection was limited to negroes, or to persons with varying proportion of negro blood, but Sights and Simon (1931) observed it in individuals of undoubtedly pure European origin. American authors have so far studied over 100 cases, but, apart from Archibald's case (1926) in the Sudan, and Russell and Taylor's (1932) and Smith's (1933, 1934) in West Africa, the literature available locally shows no record of the disease having been noticed by British or French workers in the tropics. In the course of routine blood examinations for malarial parasites and the Widal test, sickle cells have been seen, which is not surprising in a population with a high percentage of negro blood. These cases were unfortunately not studied. The striking and unusual features presented by the one here recorded therefore justified investigation and description.

The chief characteristic of the disease, even in the latent form, is the sickling of the red corpuscles, together with a varying degree of anaemia. In the acute exacerbations there may be evidence of serious illness and severe destruction of blood cells, as is well illustrated in the present instance.

The patient, L.T., was a male manual labourer, aged 24, black in colour, but with a trace of European blood. He was admitted to hospital on November 9th, 1936, with a temperature of 99.6° F., and a history of fever and generalized pains for 8 days, suggesting malaria. His organs were normal, and blood smears showed no malarial parasites but many nucleated erythrocytes. On the 15th he was discharged, against advice, with a provisional diagnosis of pyrexia. On the 24th he was readmitted with a history of fever, rigors, headache, vomiting, shortness of breath and severe epigastric pains for 4 days. His temperature was 100°, and pulse 106, small and irregular. He was a thin, anaemic, emaciated man, obviously very ill, lying prostrated and helpless in bed, and, through weakness, barely able to answer questions, though he was conscious. There was pallor, with an icteric tinge in the palms of his hands, his tongue and conjunctiva. Vomiting was persistent and intractable and bore no relation to food. His

heart showed haemic murmurs, the spleen was hard and palpable 1 inch below the costal margin, and there were scars of healed ulcers over both tibiae. On November 27th, the vomiting was less and his temperature was normal. Epigastric pain was, however, constant and severe and was his main complaint. The Widal test was negative, and repeated blood examinations showed no malarial parasites but numerous nucleated erythrocytes. In view of the obvious anaemia, a complete haematological examination was undertaken, and while estimating the reticulocyte count with brilliant cresyl blue in a moist preparation sickling of the cells was seen. His haemograms read as follows :—

Date	Erythrocytes	Leucocytes	Haemoglobin per cent.	Haemoglobin in gm. per 100 c.cm.
Nov. 26th ...	910,000	10,200	25.9	3.76
Nov. 30th ...	1,400,000	8,000	32.4	4.70
Dec. 4th ...	1,500,000	6,100	37.3	5.10
Dec. 11th ...	3,400,000	8,000	49.6	7.21
Dec. 23rd ...	2,860,000	13,600	26.9	3.92

INDICES

Date	Volume index	Colour index	Saturation index
Nov. 26th	1.2	1.4	1.1
Nov. 30th	1.3	1.1	0.8
Dec. 4th	1.4	1.2	0.8
Dec. 11th	0.8	0.7	0.8
Dec. 23rd	1.2	0.5	0.4

ABSOLUTE VALUES

Date	Corpuscular volume	Mean corpuscular volume (c. μ)	Mean corpuscular haemoglobin ($\gamma\gamma$)	Mean corpuscular haemoglobin concentration per cent.
Nov. 26th	9.0	100.0	41.7	41.7
Nov. 30th	16.0	114.0	33.5	29.0
Dec. 4th	18.5	124.0	34.0	27.6
Dec. 11th	24.5	72.0	21.0	29.0
Dec. 23rd	28.5	101.8	14.0	13.7

DIFFERENTIAL COUNT

	Nov. 27th	Nov. 30th	Dec. 2nd	Dec. 4th	Dec. 9th	Dec. 10th	Dec. 11th	Dec. 17th	Dec. 23rd
Polymorphonuclear neutrophils	82	69	60	55	53.4	33.0	36	38.7	43
Lymphocytes	7	22	27	34	26.0	53.0	57	54.0	42.3
Lymphoblasts	0	0	5	0	12.2	7.0	0	4.6	5.0
Eosinophils	0	5	0	3	4.0	2.7	4	7.0	2.5
Monocytes	0	0	0	1	0.7	0	0	0	3.2
Metamyelocytes	5	3	3	1	3.7	0	0	0	—
Myelocytes	0	0	5	6	0	3.5	2	0	2
Mast-cells	0	0	0	0	0	0.8	1	4.7	—
Myeloblasts	6	1	0	0	0	0	0	0	2

Date	Nucleated erythrocytes per c.cm.	Percentage of reticulated erythrocytes	Percentage of sickle-cell erythrocytes
Nov. 26th... ..	= 34,000	54	85
Nov. 30th... ..	= not done	60	82
Dec. 2nd	= 11,000	66	not done
Dec. 4th	= 4,800	not done	90
Dec. 9th	= 900	15	92
Dec. 10th... ..	= 1,000	not done	84
Dec. 11th... ..	= 857	8	not done
Dec. 23rd... ..	= 3,300	3	80

Polychromasia, poikilocytosis, and anisocytosis with microcytes and megalocytes, some of the latter 13μ in size, were present. Basket- and smear-cells were also seen, and Jolly's bodies were numerous. In smears taken on December 11th, only polychromasia was present, and in those of the 17th none of these conditions was visible. On the 11th his icterus index was 21, and the Van den Berg reaction was negative. Free HCl was present (4 c.cm.) in the fasting stomach. On December 27th, though still ill and weak, he demanded his discharge from hospital, and left without a known address.

COMMENTS

The two striking features in the haematological picture were the sickling of the erythrocytes and the large number of nucleated ones. The red cells were 'oats'-shape, or stellate and crescentic, with several showing fine filaments protruding from either end. The sickling, which affected as many as 92 per cent. of the erythrocytes, could be seen in moist sealed specimens as early as 4 hours

and as late as 24 hours after preparation. The presence of nucleated erythrocytes in such large numbers in the peripheral blood-stream suggested at first one of the leuco-erythroblastic anaemias, though the clinical history simulated malaria and the abdominal crisis of tabes. These cells consisted not only of those concerned in normal erythropoiesis, which are to be found normally only in the bone marrow, such as megaloblasts, early and late erythroblasts and normoblasts, but others which showed haemoglobinization of their cytoplasm with a considerable shift to the left—a true ‘megaloblastic erythropoiesis’ in Turnbull’s sense. In conjunction, therefore, with hyperplasia and excessive activity of the bone marrow, so common in the simple anaemias, there was alteration in the character of the blood regeneration, with a reversion to a primitive embryonic type of cell-evidence of the ‘pernicious’ form of the anaemia. The increase in the mean corpuscular volume and haemoglobin, as well as in the volume and colour indices, though not constant, suggests also such an anaemia. Among the abnormal leucocytes, special reference should be made to the lymphoblast. This cell, the existence of which is doubted by some, is definitely distinct in morphology from the myeloblast, and can be further differentiated by the peroxidase reaction. The high reticulocyte count (66 per cent.) is also of interest. According to Whitby and Britton (1935), in no other condition, except acholuric jaundice and a rare type of Hodgkin’s disease, is there present such a persistently high reticulocytosis. This severe damage to normal haemopoiesis, occurring periodically, would appear to suggest the action of some toxin upon an inherently imperfect erythron as a possible explanation of the etiology of sickle-cell anaemia; and some support is given to this view from the fact that the condition is a familial one and the sickle-cell tendency behaves as a Mendelian dominant. If Diggs’s (1932) statement is correct, that the disease is the most common primary blood dyscrasia of negroes in the United States, it would be of interest to determine its extent among the peoples in British and French tropical possessions.

My thanks are due to Dr. James Cook, F.R.C.S., Medical Superintendent, Colonial Hospital, Port-of-Spain, for access to the case.

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FURTHER OBSERVATIONS ON A STRAIN OF *TRYPANOSOMA GAMBIENSE*

BY

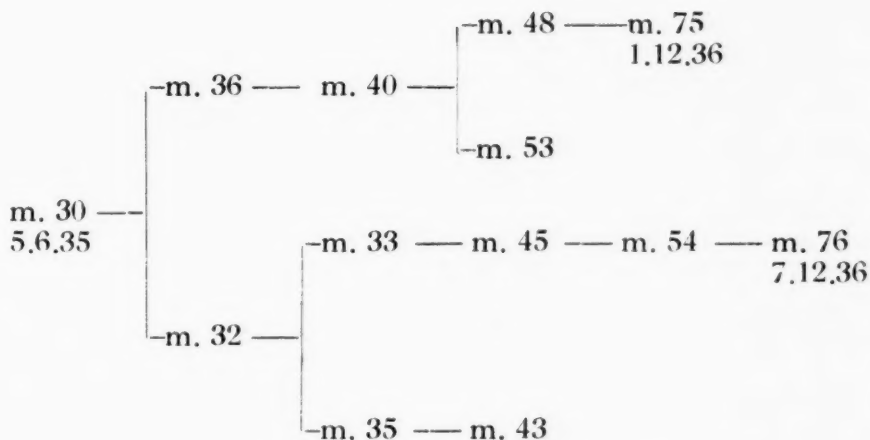
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(Received for publication 19 April, 1937)

In a previous paper (Corson, 1936) it was concluded that a strain of *Trypanosoma gambiense*, after repeated passages through monkeys and *Glossina morsitans*, showed no increase in virulence for the animals on which it was tested. Some further observations on the same strain, made during the following eight months, are given here.

The strain was maintained, as before, by passages through monkeys and *G. morsitans*, three more passages having been made, viz., from monkey 45 to monkey 54, from monkey 54 to monkey 76, and from monkey 48 to monkey 75. Two other attempts to transmit the trypanosomes from monkey 48, two from monkey 54, one from monkey 45, and two from monkey 43 failed. An additional 8th passage from monkey 40 was made, viz., to monkey 53, but an attempt to carry it further failed. The later part of the scheme of fly-passages is shown below.



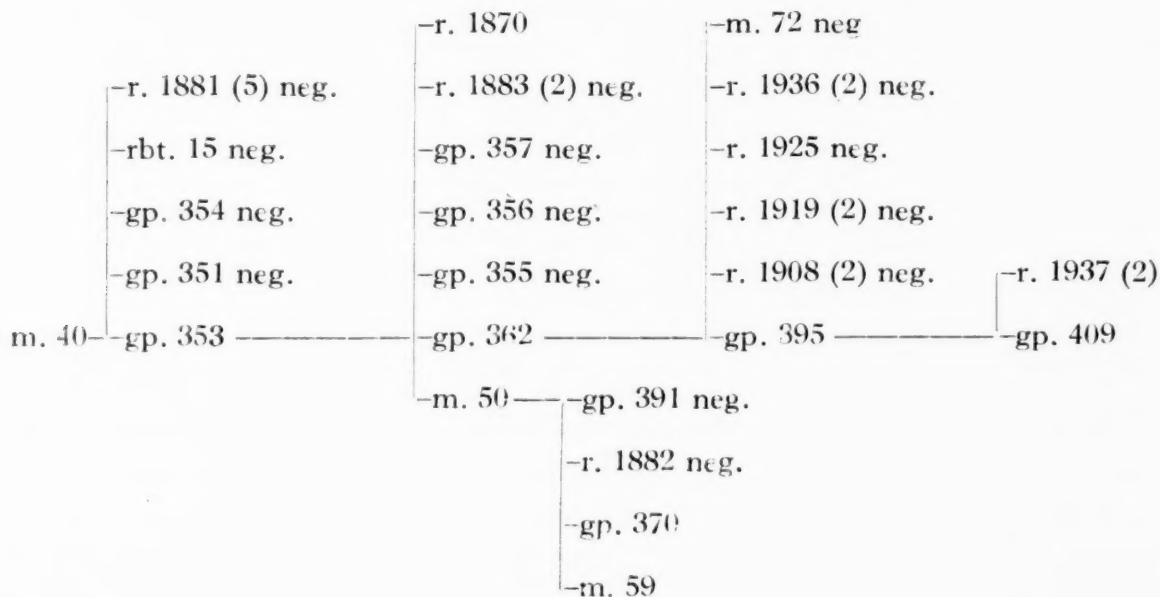
The infections in monkeys 53, 54, 75 and 76 were of the same chronic type as in the others. The transmissibility of the strain by *G. morsitans* has not apparently increased. During the last seven or eight months two monkeys have died, viz., monkeys 17 and 18. Monkey 17 was in a weak, wasted and tremulous state on November 18th, 1936, and it was chloroformed and the cerebrospinal fluid was examined. There were about 100 trypanosomes and 1,600 leucocytes in a cubic millimetre of the fluid. A thick blood film, taken at death, showed no trypanosomes in 200 fields of the microscope. Monkey 18 had also become very thin and weak by the end of 1936, and on January 20th, 1937, it was chloroformed and the cerebrospinal fluid was examined. It showed 500 trypanosomes and 800 leucocytes in a cubic millimetre. The pericardial

fluid also contained numerous trypanosomes. A thick blood film, taken at death, showed 30 trypanosomes in 100 microscopic fields.

It is well known that individuals among any species of laboratory test animal vary greatly in resistance to infection, and, although very little is known about the influence of the resistance of vertebrate hosts on the general virulence of trypanosomes, it was thought possible that changes in this strain, especially increase in virulence, might be studied best by selecting susceptible individuals among inoculated animals and transmitting their infection to others, in order to see whether such changes persisted and were general or were limited to the species or individual in which they occurred. The points to be observed were the numbers of trypanosomes in the peripheral blood, the duration of life, the presence of posterior-nuclear forms, and, possibly, the transmissibility by *G. morsitans*. It was not intended to test the effects of arsenical drugs or of human blood serum, or to try to infect man, unless other changes took place and persisted.

Among the animals inoculated from monkeys, three guinea-pigs, nos. 353, 350 and 371, appeared to be unusually susceptible, and they were selected for special observation. The schemes of transmission by inoculation from these guinea-pigs are shown in the following diagrams. The abbreviations m., gp., r. and rbt. are used for monkey, guinea-pig, rat and rabbit.

GUINEA-FIG 353



Guinea-pig 353 was mentioned in the previous paper as showing numerous trypanosomes at times and, on two occasions, posterior-nuclear forms. From the end of May to the middle of July, 1936, the numbers of trypanosomes in the peripheral blood were relatively few, but from then onwards until its death on October 4th, the 164th day, they were numerous, and posterior-nuclear forms were seen on 3 days, but in small numbers. Subinoculations failed to

infect 2 rats and 3 guinea-pigs, but were successful in guinea-pig 362, rat 1870 and monkey 50.

Guinea-pig 362 was inoculated on June 24th, 1936. Its blood, examined in stained thick films, showed no trypanosomes on July 2nd, 4th, 6th, 8th, 12th, 14th and 16th, but they were present on July 24th. The dose of trypanosomes inoculated was not small, as the blood of guinea-pig 353 on June 24th showed 2 trypanosomes per field in a thick film. Until the end of September trypanosomes were either not found or were few in the blood of guinea-pig 362, but from October until its death on January 3rd, 1937, the 191st day, trypanosomes were numerous and posterior-nuclear forms were usually present and formed from 1 to 6 per cent. of the total number of trypanosomes. The appearance of thin blood films was similar to what is common in infections with *T. rhodesiense*. Subinoculations, however, gave no indications of generally increased virulence, as 7 rats and a monkey failed to become infected, although large doses of trypanosomes were given.

Rat 1870 was inoculated from guinea-pig 353 on May 19th, 1936, when a thick film of the guinea-pig's blood showed 15 trypanosomes per field. Thick blood films of the rat showed no trypanosomes on May 23rd, 26th, 28th and 30th, but 1 trypanosome was seen in 100 fields on June 29th, 8 on July 2nd, 7 on July 4th, and 1 on July 5th, since when no trypanosomes have been seen, although the blood was examined in thick films on nine more days in July, twice in August, once in September, twice in October, on ten days in November, three times in December, twice in January, and three times in February. The rat is apparently in good health and seems to have recovered. Its blood was inoculated into a rat on January 19th, 1937, and no infection has yet appeared.

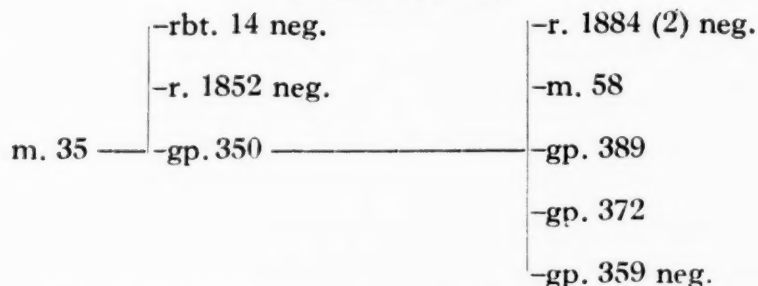
Guinea-pig 395 was inoculated from guinea-pig 362 on October 25th, 1936, and trypanosomes were seen on November 2nd—an unusually short incubation period. They were present in moderate numbers until the end of November, when they became numerous, and posterior-nuclear forms were first seen on November 28th, forming 4 per cent. of the trypanosomes in a thin film. Trypanosomes remained numerous during December and early January, and posterior-nuclear forms were seen in two out of five examinations in December. The guinea-pig died on January 11th, 1937.

Two rats, no. 1937, were inoculated from guinea-pig 395 on December 18th, and the incubation periods were 25–29 days and 30–31 days. The infection has been of a chronic type in both rats, thick blood films showing few or no trypanosomes.

Guinea-pig 409 was inoculated from guinea-pig 395 on January 1st, 1937, and trypanosomes appeared 9 days later. The infection has so far been of a mild character.

The infections in monkey 50, guinea-pig 370 and monkey 59 have been chronic.

GUINEA-PIG 350



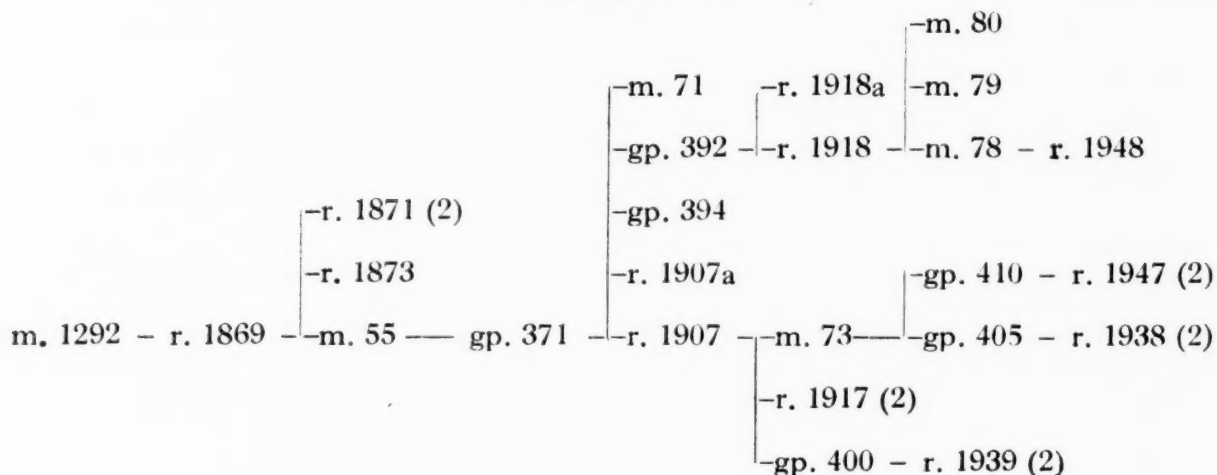
Guinea-pig 350 was inoculated from monkey 35 on April 15th, 1936, and the course of the disease up to the beginning of July was shown in the previous paper. Trypanosomes were more numerous in the blood from July onwards until its death on October 7th, 1936, the 94th day. Although the numbers of trypanosomes in thick films were often 20 or 30 in a field, no posterior-nuclear forms were ever seen in the examinations of thin films.

Guinea-pigs 372 and 389 were inoculated on July 30th and October 4th respectively, days when trypanosomes were numerous in the blood of guinea-pig 350. Trypanosomes were first seen in the blood of guinea-pig 372 on November 28th, and were afterwards present in small numbers. The guinea-pig died on January 16th, 1937, almost certainly from an acute infectious disease that was prevalent among the guinea-pigs at the time. Guinea-pig 389 first showed infection on November 6th, and the numbers of trypanosomes remained small until the end of December. They then increased, but the guinea-pig died on January 16th from the prevalent infectious disease.

Monkey 58 was inoculated from guinea-pig 350 on July 30th, 1936, when there were numerous trypanosomes in the guinea-pig's blood. Thick blood films of monkey 58 showed no trypanosomes in 200 fields on August 4th, 7th, 9th, 12th, 17th, 20th, 26th, 28th, September 2nd and 18th, but they were found on the next examination on November 27th, and have been present in small numbers since.

The virulence of the trypanosomes shown in guinea-pig 350 seems to have been relative to that animal only among those inoculated.

GUINEA-PIG 371



On the death of the original monkey, no. 1292, from Uganda, a rat, no. 1869, was infected by inoculation of the monkey's cerebrospinal fluid, and the strain was maintained by further inoculations in order to compare it with the infections transmitted by *G. morsitans*. Trypanosomes were last seen in the blood of rat 1869 on October 2nd, 1936, and it died on December 18th, the 230th day after inoculation. Between October 2nd and December 18th, 200 fields of thick blood films were examined on 17 days. In rats 1871, 1871a and 1873 also the disease was of a very chronic character.

Monkey 55 was inoculated from rat 1869 on June 27th, 1936, and its blood showed trypanosomes at the first examination on July 6th. They were present at almost every examination up to the end of August in small or moderate numbers, and afterwards they were very few or absent in 200 fields of thick blood films.

Guinea-pig 371 was inoculated from monkey 55 on July 30th, 1936, and lived for 107 days afterwards. Trypanosomes were not seen in its blood on August 6th, but were present in moderate numbers on August 9th. They then remained few in number until the end of September, when they increased up to 10 or 20 per field of a thick blood film. Posterior-nuclear forms were seen on one day only, November 5th, when they formed 1 per cent. of the trypanosomes of a thin blood film.

Guinea-pig 392 was inoculated from guinea-pig 371 on October 8th, 1936, and its blood showed trypanosomes at the first examination on October 17th. They increased in number, and the infection ran a more acute course than usual. Posterior-nuclear forms were seen on two days, November 12th and 25th, forming 2 and 1 per cent. respectively of the total number of trypanosomes. The guinea-pig died on January 9th, the 93rd day after inoculation.

Rats 1918 and 1918a were inoculated from guinea-pig 392 on November 18th, and both became infected, with incubation periods of 10-12 and 15 days. In the blood of rat 1918a trypanosomes were always very few or not seen, but they were usually present and sometimes numerous in rat 1918. No posterior-nuclear forms were seen. Rat 1918a died on January 31st, 1937, the 74th day after inoculation, but no trypanosomes had been seen in its blood since December 21st, and the cause of death is doubtful. At the beginning of February, 1937, the trypanosomes became very numerous in the blood of rat 1918, thin films showing from 20 to 30 per field. They have remained very numerous throughout February, and are mostly of slender shape, sometimes showing a large amount of volutin material. No posterior-nuclear forms have been seen. In a single test with normal human serum *in vitro* the trypanosomes showed greater resistance than a strain of *T. rhodesiense* tested with the same serum, the number being only reduced to one-tenth after 24 hours at 37° C. The blood of rat 1918 was inoculated into monkey 78 on February 19th, and into monkeys 79 and 80 on February 21st. In all three monkeys trypanosomes were present on the 2nd day after inoculation. A rat, no. 1948, was inoculated from monkey 78 on

February 22nd, and trypanosomes appeared in its blood on the 7th day.

Guinea-pig 394 was inoculated from guinea-pig 371 on October 25th, and showed no infection for 16 days. The infection took a chronic course, thick blood films showing either no trypanosomes or very few. It died, however, from the prevalent infectious disease on January 14th, 1937.

Rats 1907 and 1907a were also inoculated from guinea-pig 371 on October 25th. One of them, rat 1907, showed trypanosomes on the 12th day, but none was found in rat 1907a until the 31st day after inoculation. The infection in rat 1907 was as acute as with some strains of *T. rhodesiense*, and the rat died on the 69th day with its blood swarming with trypanosomes. Except on the day of its death, the numbers of trypanosomes counted in thick blood films varied from about 3 to 20 per field. When examined in thin films they were seen to be mostly slender, with a free flagellum and a large amount of volutin material. No posterior-nuclear forms were seen. In rat 1907a the infection was chronic, few or no trypanosomes being seen in thick blood films.

Monkey 71 was inoculated from guinea-pig 371 on October 27th, 1936. Its blood was negative on October 31st, but trypanosomes were seen at the next examination on November 4th. The infection has shown the chronic character that is usual in monkeys infected with this strain.

Monkey 73 was inoculated from rat 1907 on November 18th, and the incubation period was 10–12 days. Trypanosomes have since been present in small or moderate numbers at nearly every examination.

Guinea-pig 400 was inoculated from rat 1907 on November 18th, and the incubation period was 15–16 days. The infection has run a rather acute course, with considerable variations in the numbers of trypanosomes in the blood. No posterior-nuclear forms have been seen.

Rats 1917 and 1917a were inoculated from rat 1907 on November 18th. The incubation period in both was 13–14 days, and the infection has been of a very chronic nature.

Guinea-pig 405 was inoculated from monkey 73 on December 6th, and the incubation period was 7–8 days. The trypanosomes rapidly increased in number, a thick film on December 26th showing 40 to 50 trypanosomes per field. On December 28th the guinea-pig was very ill, and its blood was inoculated into two rats, no. 1938. The guinea-pig died on December 29th, the 23rd day after inoculation. The presence of infectious disease among the guinea-pigs affects the significance of this acute course. The two rats, no. 1938, showed infection after incubation periods of 9–10 and 11–12 days, and the disease has been very chronic.

Guinea-pig 410 was inoculated from monkey 73 on January 7th, 1937, and trypanosomes were first seen in its blood on January 25th. The infection has so far been moderately acute. Its blood was inoculated into two rats, no. 1947, on February 6th, when trypanosomes were very numerous in the guinea-pig's

blood. Both became infected, with an incubation period of 10-11 days, and the infection has been of a very chronic character.

Rats 1939 and 1939a were inoculated from guinea-pig 400 on January 5th, and both had an incubation period of 9 days. The infection has been chronic.

Observations on this series of animals infected originally from guinea-pig 371 will be continued, monkeys, guinea-pigs and rats being inoculated in turn, so as to avoid, if possible, a specialization of the trypanosomes for one kind of host.

TRANSMISSIBILITY OF THE STRAIN BY *G. MORSITANS*

Attempts were made to transmit the strain by *G. morsitans* from guinea-pigs 350, 362 and 371, and from monkeys 55 and 71, without success. No conclusions can be drawn from these failures.

SUMMARY AND COMMENTS

Some further observations on a strain of *T. gambiense* were made, in order to see whether the strain would acquire increased general virulence and come to resemble *T. rhodesiense*. Three guinea-pigs which showed unusual susceptibility to the infection were selected from among the animals, which were inoculated from monkeys, and further inoculations into monkeys, guinea-pigs and rats were made from these guinea-pigs.

In monkeys the infection never showed any similarity to infections with *T. rhodesiense*, and the same is true for most of the guinea-pigs and rats; but in guinea-pig 362 and rat 1907 there was some similarity. In guinea-pig 362 the infection developed more slowly than is usually seen in infections with *T. rhodesiense*, but in the later months the numbers of trypanosomes and the presence of posterior-nuclear forms gave an appearance in thin films that was indistinguishable from that of an infection with *T. rhodesiense*. In rat 1907 the infection was as acute as an infection with *T. rhodesiense*, but the continued absence of posterior-nuclear forms is at least rare in the latter infection when trypanosomes are numerous. In neither case, however, was the increase in virulence passed on to monkeys or rats, as would occur, so far as is known, with *T. rhodesiense*; and it cannot therefore be thought that a general increase in virulence had developed.

There has been an opportunity of comparing this strain with a not very virulent strain of *T. rhodesiense* that has been maintained in sheep for over two years by transmission by *G. morsitans*. About 100 rats have been inoculated with this strain from sheep and other animals and from man, and about 50 rats have been infected by the bites of single isolated flies. The infections in the rats have been usually acute, and some shift of the nucleus forwards or backwards with fairly numerous posterior-nuclear forms was present in nearly all

cases. In one or two instances great resistance to the infection was shown, the rat living for about three or more times as long as the average duration; but when its blood was inoculated into other rats they got the ordinary acute infection, showing apparently that the strain of trypanosomes had undergone no real or stable change in virulence for rats. This may be similar to the human case reported by Lamborn and Howat (1936).

It is difficult to draw conclusions from these observations on one strain of *T. gambiense*, but the impression is given that, whatever may be the relationship between *T. gambiense* and *T. rhodesiense*, a typical strain of either does not easily change so as to resemble the other. It may be that different strains of one species (using the word species for convenience) also retain their mild or virulent character firmly, but there are instances of mild strains of *T. brucei* developing increased general virulence and retaining it.

The history of trypanosomiasis in East Africa, though lacking detail, also suggests that the three species of polymorphic trypanosomes do not, at least easily, change so as to resemble one another. In Uganda all the strains of *T. gambiense* recorded in the early reports of the Sleeping Sickness Commission of the Royal Society in the years 1903, 1904 and 1905 showed little virulence for laboratory and domestic animals, yet *T. brucei* seems to have been present at this time. The 'Jinja trypanosome' found in cattle in 1903 and in 1909 was regarded as *T. brucei*, and the trypanosomes from wild flies used in an experiment with the serum of reedbuck 2359 by Duke (1913) killed white rats in 21, 23 and 25 days, while another strain from wild flies killed a rat in 54 days. In German East Africa also (except on the Rovuma River) there is no record of any human infections resembling *T. rhodesiense* in their pathogenicity to animals, though it seems to be practically certain that *T. brucei* was also present. The three Tanganyika strains of *T. gambiense* sent by Kleine in 1912 to Bruce in Nyasaland showed very low virulence for monkeys, guinea-pigs and rats, though more for dogs, while the cases of sleeping sickness in Nyasaland at that time seem to have been all infections with *T. rhodesiense*, resembling closely, in man and experimental animals, the cases described by Kinghorn and Yorke (1913) in Northern Rhodesia and those occurring at present in Tanganyika Territory. In Zululand *T. brucei* has existed for many years, but no case of human trypanosomiasis has hitherto been found there. It is difficult to explain the occurrence, found since the Great War, of cases of sleeping sickness on the West Coast of Africa infected with trypanosomes indistinguishable from *T. rhodesiense*. Lester (1933) regards them as modifications of *T. gambiense* and considers that the two human trypanosomes are identical. There also chronic human trypanosomiasis and nagana due to *T. brucei* have existed together for many years.

There are good reasons, apparently, for regarding the three polymorphic trypanosomes as forming one species for purposes of classification; but it is not yet known whether any of them can change into either of the other two, a question that has been very much discussed during the last 25 years.

The best way of attacking these problems seems to be by laboratory experiments.

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THE INFECTIVITY TO MAN OF A STRAIN OF *TRYPANOSOMA RHODESIENSE* TRANSMITTED THROUGH SHEEP BY *GLOSSINA MORSITANS*, AND ITS RESISTANCE TO HUMAN SERUM *IN VITRO*

BY

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(*Received for publication 7 May, 1937*)

In October, 1934, Corson isolated a strain of *T. rhodesiense* by feeding a box of laboratory-bred *G. morsitans* on an untreated case of sleeping sickness; and he has since maintained this strain in the laboratory in sheep alone, transmitting the infection by cyclically infected *G. morsitans*. In March, 1936, 16½ months after isolation, he showed (Corson, 1936) that the strain was still infective to man, by feeding an infected fly on his arm. This infection corresponded to the 7th animal passage of the strain after isolation from man.

ACTION OF HUMAN SERUM

Using the *in vitro* technique of Warrington Yorke, Adams and Murgatroyd (1930), the reaction to human serum of the strain from the 6th animal passage onwards was investigated. At each animal passage a number of rats was inoculated by syringe from the cyclically infected sheep, while a couple of rats, infected directly by the bites of tsetse-flies, were also examined to see if the action of the serum was influenced by the method of infecting the rats. The transmission of the strain, with the rats infected at the various passages, is shown in the transmission chart (p. 286).

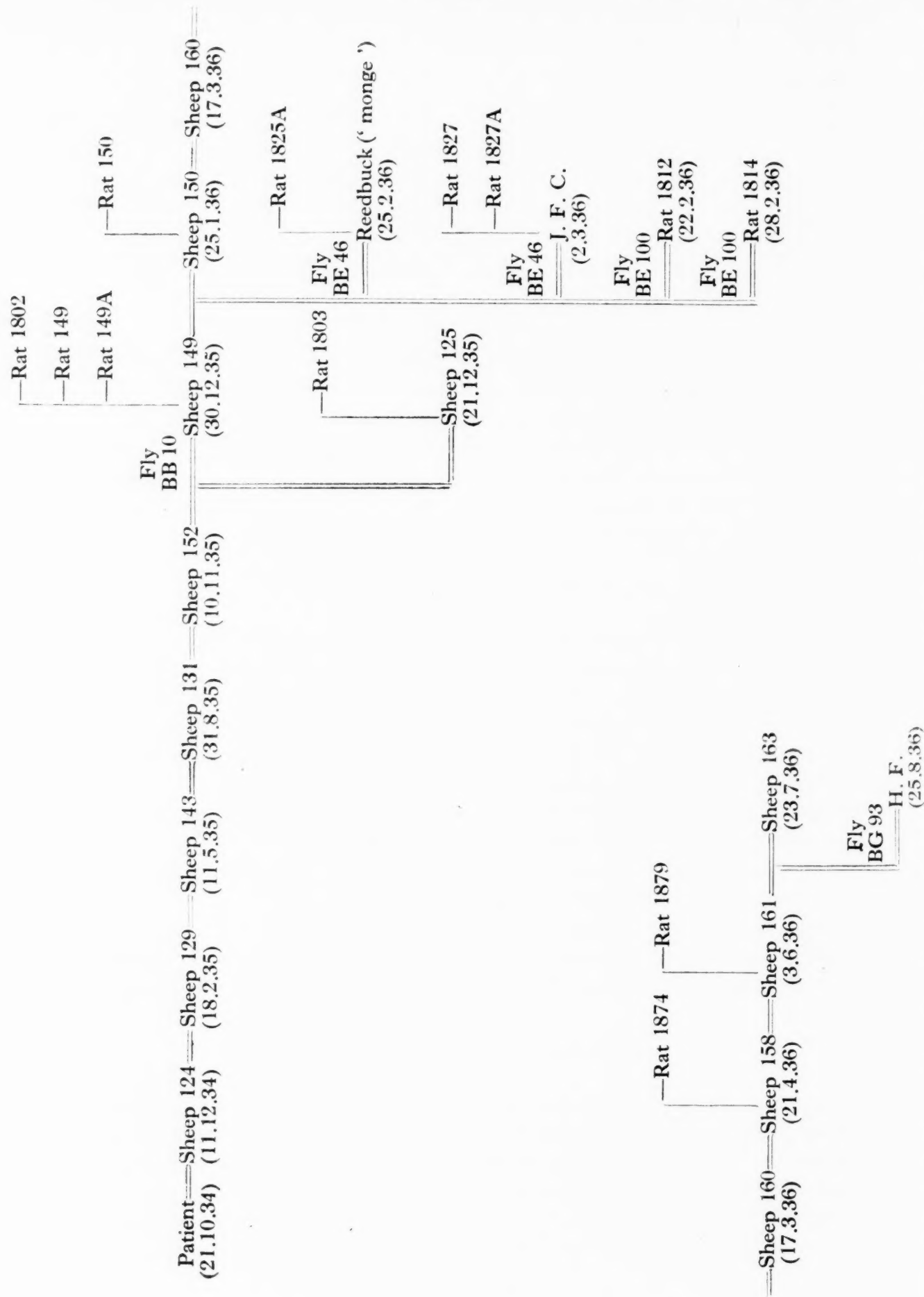
Table I (pp. 288-89) shows the action *in vitro* of normal human serum on the strain at the various animal passages.

Comparing the experiments with rats 1812 and 1814 with the others, it would seem that infecting the rats directly by feeding flies produces no difference in the *in vitro* action of serum as compared with rats infected by syringe.

INFECTIVITY TO MAN

The serum experiment, using rat 1879, having been started on the morning of August 25th, 1936, an infected tsetse-fly, BG 93, which was one of the box of flies used to transmit the strain to the 11th passage sheep no. 163, was fed on the author's forearm that afternoon (i.e., 24 hours after blood had been withdrawn from a vein to get the serum for the experiment). At 11 a.m. on September 1st (i.e., 7 days later), there was a feeling of general malaise and chilliness, with

TRANSMISSION OF THE STRAIN OF *T. rhodesiense* USED IN THE EXPERIMENTS



The dates in brackets show when flies were first fed on the men or animals. Double lines (==) indicate infections by cyclically infected *G. morsitans*, and single lines (—) indicate infections by syringe.

a temperature of 98.6° F.; and at 11.30 a.m. 5 rats were inoculated, each with 1 c.cm. of blood from a vein and 1 c.cm. normal saline mixed. At 8.30 p.m. that day the temperature was 102° F., and a single trypanosome was found in a stained thick film after prolonged search. Next morning (the 8th day) a single trypanosome was again seen after 126 fields of a stained thick film had been searched, and treatment was then started with Bayer 205. The attack was one of normal severity, with a maximum temperature of 104.6° F. on the evening of September 2nd, and followed a normal course under treatment with 5 one-gm. doses.

Of the 5 rats inoculated, 3 became infected, with incubation periods of 10, 16-18 and 22-24 days respectively, while the other 2 were still negative on the 26th and 27th days. It would thus seem that at the time when the rats were inoculated the trypanosomes must have been very scanty in the blood (or that the shed blood, when inoculated into the rats, may have had some action on the trypanosomes present).

COMMENTS

This experiment has shown that a strain of *T. rhodesiense*, transmitted through sheep by cyclically infected *G. morsitans*, is still infective for man 22 months after having been isolated. The two infections produced 16½ months and 22 months after isolation showed no diminution in virulence of the strain, and were no different from the type of infections produced by strains of *T. rhodesiense* which had been maintained by syringe inoculation in sheep and goats for 19 months (Corson, 1932) or in rats for 11 months (Fairbairn, 1933).

Despite the fact that at the 9th passage the trypanosomes were susceptible to 1 sample of the author's serum, and at the 10th passage to 6 samples of serum, yet an infection developed when an infected fly was fed on him. This appears to confirm previously expressed opinions that the action of serum, *in vivo* and *in vitro*, on a strain is no criterion of the ability of that strain to infect the host furnishing the serum (Mesnil and Leboeuf, 1912; Collier, 1924; Corson, 1932; Adams, 1933; and Fairbairn, 1933). With regard to human serum, it would almost appear that so long as a strain of *T. rhodesiense* is resistant to any human serum it is capable of infecting any man.

Adams (1933) stated that in each of three strains of *T. rhodesiense* 'definite loss of resistance to normal human serum *in vitro* was observed after a single fly transmission, and, in the case of Strain A, a second fly transmission apparently caused further loss of resistance, although this loss may have occurred to some extent in the intermediate guinea-pig host.' In a previous experiment (Fairbairn, 1933) the strain recovered from man, after experimental infection, appeared to have the same reactions to human serum *in vitro* as the parent strain isolated from the sleeping sickness patient 11 months previously. If this single experiment is generally accepted, then the examination of the trypanosomes in rats

TABLE I

Showing the action, *in vitro* at 37° C., of normal human serum on a strain of *T. rhodesiense* at various animal passages

Date of experiment	Rat				No. of days since strain was isolated from man	Serum undiluted of	Trypanosomes per sq. mm. (Thoma-Zeiss)								At 24 hours rat inoculated with serum-trypanosome mixture
	No.	Inoculated from	Passage	Inoculation period in days			Start	1	2	4	6	8	12	24	
12.3.36	1802	6th passage sheep no. 149	1st	6-9	26	H.F. Saidi Tuga	66	65	72	47	-	5	-	0	Rat neg. 30 days
					508			53	52	39	-	8	-	0	" " 30 "
								56	43	12	-	0	-	0	" " 30 "
19.4.36	149	"	1st	6	39	H.F. Mwambura Zaid Selemani Kitambi	60	11	11	1	-	0	-	0	" + 14 "
					546			10	3	0	-	-	-	0	" neg. 61 "
								4	0	0	-	-	-	0	" + 15 "
								9	13	1	-	0	-	0	" neg. 61 "
								5	0	0	-	-	-	0	" " 61 "
5.5.36	149A	"	1st	6	55	H.F. Benedicto Saidi	110	90	68	31	5	2	1	0	" + 8 "
								70	58	27	8	1	2	0	" + 8 "
								60	40	4	0	0	-	0	" neg. 45 "
								80	42	3	0	0	-	0	" " 45 "
								68	48	6	0	0	-	0	" " 45 "
6.3.36	1803	6th passage sheep no. 125	1st	6-9	20	H.F. E.B. Saidi	60	31	11	5	0	-	-	-	" + 5 "
								30	19	4	0	-	-	-	" neg. 35 "
								18	16	4	0	-	-	-	" " 35 "
19.4.36	150	7th passage sheep no. 150	1st	10	41	H.F. Mwambura Zaid Selemani Kitambi	72	110	78	21	-	0	-	0	" " 33 "
								106	82	46	-	0	-	0	" " 61 "
								78	36	0	-	-	-	0	" " 61 "
								160	78	7	-	0	-	0	" + 14 "
								72	24	0	-	-	-	0	" neg. 19 "

1827 and 1827A, recovered from a man experimentally infected 16½ months after the strain was isolated from the patient, would appear to show that the original strain could not have had a marked degree of serum resistance. The subsequent degree of serum resistance after various fly transmission can roughly be expressed as follows :—

TABLE II

Strain	No. of experiments	No. of sera tested	No. of tests positive
Strain recovered from man infected 16½ months after isolation (corresponding to the 7th animal passage)	2	6	1
Strain from 6th passage animals	4	16	5
" " 7th " "	4	14	3
" " 9th " "	1	9	0
" " 10th " "	1	10	1

Comparing this strain, which had been transmitted solely by tsetse-flies, with the other strain (Fairbairn, 1933), which had been transmitted by syringe, it would not appear that the fly transmissions had had any influence, one way or the other, on the action of human serum *in vitro*.

The relationship of *T. rhodesiense* and *T. brucei* is still the subject of discussion. This experiment has shown that after 22 months' maintenance in animals a strain of *T. rhodesiense* has still retained its power to infect man, and has retained its resistance to human serum *in vitro* to a considerable extent. It has, in fact, shown no tendency as yet to revert to a *T. brucei* type. With the knowledge that is now accumulating that transmissions by syringe inoculations are almost comparable, at least in the early years, to transmissions by cyclically infected tsetse-flies, it would again be urged that the numerous strains of *T. rhodesiense*, which are at present being maintained in European laboratories, should be inoculated into human volunteers to test their infectivity to man. If they are proved to be still infective to man, it will immediately show for how many years this character has been maintained. On the other hand, should some or all of the strains be non-infective, then further investigation would be directed to finding out after how many years this infectivity had been lost, and correlating it with the loss of other characteristics, such as polymorphism, posterior-nuclear form production, and transmissibility by tsetse-flies.

SUMMARY

1. A strain of *T. rhodesiense*, which had been transmitted by cyclically infected *G. morsitans* through sheep, was still infective to man 22 months after isolation.

2. Cyclical transmission of the strain did not appear to have any influence on the action of human serum *in vitro* on the strain, especially when compared with a similar strain which had been transmitted through rats by syringe inoculation.

3. It is urged that strains of *T. rhodesiense* maintained for years in European laboratories should now be inoculated into human volunteers, as such inoculations would give immediate data as to the number of years over which *T. rhodesiense* remains infective for man.

ACKNOWLEDGEMENTS.—I desire to acknowledge my thanks to Dr. J. F. Corson, Research Laboratory, Tinde, for laboratory facilities, for permission to examine this strain, and for his help in the diagnosis and treatment of the infection.

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STUDIES IN RURAL HYGIENE IN THE TROPICS

BY

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V.—THE PREVENTION OF LEPROSY

(Received for publication 20 May, 1937)

During a visit to the East, several opportunities presented themselves of seeing the work in progress with regard to leprosy.

At Penang, Dr. Eveson took me to Pulau Jerejerak Leper Settlement and demonstrated the notable efforts being made on the island to cope with this disease. At the Quarantine Station every precaution was employed to diagnose early cases of leprosy among the coolies so that they might be sent back to India.

The treatment of nodular and nerve cases of lepers by yaws inoculation (Plate V, fig. 1) appeared to be causing marked improvement. Even during the incubation period of the yaws the leprosy nodules began to shrink and the affected skin to lose its redness. The method is based on the observation that the Malays, large numbers of whom have had yaws, contract leprosy much less frequently than do the Chinese and the Tamils, who have not been much infected with yaws. Dr. Ryrie, who is in charge of leprosy work in Malaya was, at the time, in Australia, and Dr. Verasingham was carrying on the experimental treatment. As only volunteers are treated by this method, there may be an opportunity here of settling, by experimental means, part of the yaws-syphilis controversy.

While I was at Kuala Lumpur, Dr. R. B. MacGregor accompanied me to the mainland leper settlement of Sungei Buloh, 15 miles from Kuala Lumpur, where some 1,500 lepers are collected—Chinese, Tamils and Malays; Dr. Hughes was in local charge, with a qualified matron. The medical officers' and the matron's quarters were on elevated ground, which gave the advantage of a good breeze and, incidentally of a very fine view.

At Sungei Buloh there is land available for extension of the village type of leper settlement; at present, besides the hospital accommodation there are numbers of single- and married-quarters and also six-bed male and six-bed female quarters. Even the prison accommodation is excellent and apparently much to the liking of the few inmates seen. The beautiful site, in a wide open valley, is one which, it appeared, might be hot; but I am informed that the patients did not usually complain of the heat, but rather of the cold—perhaps, as Dr. Hughes suggested, owing to some dissociation of sensation.

Treatment of many kinds is given here, and new drugs are tried experimentally; it was demonstrated how trypan blue and fluorescin given

intravenously appear to have a selective action on the nodular leprosy lesions and stain them. The Chinese patients, who are in the majority, collaborate readily as volunteers in trying new remedies.

Some caution was expressed here as regard cures, owing to experience of unexpected relapses. While it was regarded as probable that early cases were curable, it was pointed out that certain older cases, formerly stated to be cured, and actually demonstrated as cured, subsequently relapsed after long periods.

Work in the settlement is voluntary, and a small payment has to be given, using a local currency, for any work done. There is a regular internal shop-keeping business on a small scale carried on by the patients themselves, who sell tobacco, cigarettes, sweetmeats and drinks of various kinds. As yet there are, however, no organized village industries, building or farming, but it is anticipated that these will be brought into existence presently. The servants of the officers, the Sikh constabulary, and the orderlies betray no fear of the infection.

In Calcutta, at the All-India Institute of Hygiene and Public Health, I met Dr. Lowe who is in charge of leprosy research there; he kindly took me round the Institute and introduced me to members of the staff.

At Mandapam, Dr. Deutrom, the Medical Superintendent of the Quarantine Station, informed me that during the year no less than 160 lepers had been discovered in transit there and turned back.

In Colombo, I met Dr. Cochrane, who was proceeding to India for a period of years to study leprosy control. Dr. Cochrane says that he finds it possible to diagnose leprosy in such an early stage that the usual signs may not yet have appeared and in the absence of the discovery of the leprosy bacillus. Cases which can only be diagnosed clinically in this manner by a specially trained observer are stated to be by no means rare among young people, and they may recover quite spontaneously. Such a statement by a well-recognized authority is of great significance. It means that the figures of leprosy obtained in any survey by the use of the accepted diagnostic methods fail to represent the real total of lepers existing.

Since leprosy is such a widespread disease in our tropical Empire, it is perhaps worth while noting the direction in which policy tends. When I first went to West Africa in 1914, there was in one Colony an institution called the leper asylum; in this a few unfortunate individuals, who had been picked out, more by chance than anything else, as dangerous from the infective point of view, were incarcerated. Since their existence was painfully monotonous they always wished and often tried to escape; as far as possible they were prevented from doing so by guards and barbed wire. This crude type of incarceration of lepers is, let us hope, fast dying out. Another control, less barbaric than the first, is effected by placing lepers in settlements on islands from which they cannot hope to escape. Such an island is Pulau Jerejerak, already mentioned, near Penang. The leper settlements on this beautiful island are very well administered and excellently supervised. The treatment, diet and hospitalization

are of a high order. But there appeared to be one serious drawback to a successful solution of the leprosy problem, or rather of the problem of leprosy individuals. Between the steep hills and the sea there was a mere strip of ground (Plate V, fig. 2) where there was no room for spreading the patients out on farms or in small village communities. Such activities as one saw—for example, schooling, exercises by troops of boy scouts, musical instruction and so on—could have been equally well or even better carried on in ampler space. As an instance of the scarcity of room, a football-ground was being made by reclamation of a portion of seashore. However well equipped and administered an island settlement like this may be—and Pulau Jerejerak was eminent in these respects—it hardly appears to meet the requirements of the case; some area less cramped seems desirable, in order that the various activities of individual leper patients may have fuller scope.

The next stage of incarceration may be represented by that on a mainland site, like Sungei Buloh, where, as we have seen, there is ample land available for extension and for the organization of farming and village industries of all kinds. It may be remarked that here the degree of actual restraint did not seem such as would prevent a really determined patient from escaping to the jungle, if he made up his mind to do so.

This brings us to the consideration of a still further development which appears to deserve a full trial. That is, having established a mainland settlement such as this, which serves the needs of a large area of a well-controlled country, to do away altogether with the principle of incarceration in it and to observe the results.

It has to be remarked that in practically no country in the tropics is even a majority of the existing lepers collected in any leper asylums or settlements. Chiefly owing to the lack of adequate medical staffs, and to the consequent absence of thorough examination of the people, the leprosy incidence in tropical countries is difficult to ascertain. Leprosy is one of those diseases which is very apt to be overlooked in any community, as is shown by the results of every new survey which is undertaken with the specific object of detecting lepers.

For example, the Annual Medical and Sanitary Report of Sierra Leone for the year 1926 recorded 43 lepers as patients at the Colonial Hospital. Yet in the next two years, when, at the request of the Government, I undertook a general survey of diseases in the Protectorate, I discovered a total of 243 cases of leprosy among just over 8,000 persons examined. This figure would have probably been higher had the investigation been a special survey for leprosy cases; and it would certainly have been so if the type of case referred to above as recognizable only by clinical appearance could have been included.

Therefore, it is often self-deception to assume that the leprosy problem is being solved by isolating a small percentage of the total lepers against their will. Inside the settlement this feeling of being imprisoned is bound to react on the behaviour of the inmates, and actually does so in various ways. For

example, they have to be paid for their work and it is even then done rather reluctantly. Many of them comply with the conditions imposed in a grudging manner, as if they were under the impression that they would be better off if only they could regain their liberty.

The advantage of having available a mainland settlement of large size, such as Sungei Buloh, is that in such a place a practical and useful experiment could be made to decide the value of an entirely voluntary institution. There is or could be here ample space for providing for lepers in regard to their housing, family life and useful occupation and recreation, not to mention the treatment of their disease. Thus the lives of the patients could be so arranged for their convenience, comfort and interest that they might prefer to stay inside the settlement than to go elsewhere.

Under this voluntary system, if they got tired of the life in the settlement no one would prevent them from going away; but when they left they would come under surveillance and be required to report regularly to the nearest medical officer or assistant in their neighbourhood. But it might well prove to be the case that they would soon find the life of the village, with its poor resources, limited occupations and lack of entertainment, very much less attractive than the settlement, and that they would return there even more willingly than they had left it.

If such an entirely voluntary system is to be tried—and it seems very desirable that it should be given a trial as an experiment—I believe that it will be necessary to make the acceptance of treatment also entirely optional. But in view of the satisfactory voluntary response of the patients to any promising form of treatment, and also of the fact that the untreated patient is not likely to infect healthy people so long as he or she remains contentedly in the settlement, it is difficult to see any insuperable objection to making acceptance of treatment voluntary.

Leprosy, contracted often in early youth, of long incubation and slow development, is so commonly a chronic affection that provision for the daily life of the patient presents a problem just as great as the care of the disease itself. Slightly or even grossly infected persons have a chance of spontaneous recovery if they survive long enough. They may be cured by treatment, but here again it may be a slow and often unsatisfactory process, and for a very extended period the leper's prospects of ultimate recovery may be uncertain. Therefore, our first endeavours ought to be fully to realize the tedium of the leper's life, and our next to make provision to relieve it in every possible way.

Since there is in Sungei Buloh an unlimited supply of material, in the form of patients in all stages and with various types of the disease, and since the effects of leprosy on three entirely different races, Malays, Chinese and Tamils, can be studied, a leprosy research centre might with advantage be established here. A team of selected workers, adequately endowed and prepared to work in co-operation for a period of years in a settlement such as this, would be in a position

to make most valuable additions to knowledge and important discoveries concerning the disease.

No one can doubt the necessity of making leper settlements attractive to the greatest possible number of sufferers, so that these may be induced to remain in them for as long as they are deemed infective and a potential source of danger to others. But there is a much more fundamental matter than the state of the settlement to be considered: the state of the large rural area in the hinterland is the real difficulty. In leprosy we have a problem of great magnitude, and it seems that, unless some more far-sighted policy as regards prevention is reached and put into effect, the propagation of leprosy in the villages may continue indefinitely.

It is unfortunately a fact that many native peoples do not fear lepers: they treat them in exactly the same way as they treat healthy people; they neither segregate them from the village nor do they even forbid their children to associate with them. In villages in Africa I have frequently seen a leper, with leprosy nodules all over his face, surrounded by a group of little children and playing with them. The children showed no fear of touching him, nor did their parents appear to consider it necessary to warn them of any danger.

In such circumstances it is unlikely that any great progress will be made in preventing the spread of infection until people are themselves convinced of the danger of contact with lepers. While it will doubtless be possible in time to create a public opinion in favour of the general sanitation of villages and rural dwellings, it is likely to be more difficult to convey instruction for the control of such diseases as leprosy and tuberculosis. It is true that periodic visits of medical men and sanitary inspectors usually serve to stir up interest, and even a show of enthusiasm, on the part of the villagers for a brief time; but in order to produce a less transient effect more systematic methods will be required. Only in this way shall we escape being the subjects of the unconscious irony which appeared in the essay of a West African schoolboy on sanitation, who wrote: 'In many countries of the world, especially the British Empire, sanitary services have been started, and by the spasmodic efforts of the authorities many lives have been redeemed.'

The solution of the problem of village sanitation and the prevention of many infectious diseases, such as leprosy, appears to lie in thorough education of the people in healthy living. Any scheme for such education, if it is to be effective, will necessitate a great extension of the present teaching facilities, and the inclusion in all school curricula of training in personal, domestic and village hygiene.

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EXPLANATION OF PLATE V

- Fig. 1. Primary yaws lesions (one-and-a-half months old) in treatment of leprosy.
- Fig. 2. Pulau Jerejerak. View of new settlement, showing the limited area between the hills and the sea.



Fig. 1



Fig. 2

H. R. Grubb, Ltd., Poplar Walk, Croydon.



NOTES ON ETHIOPIAN SIMULIIDAE.—I

BY

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(Received for publication 24 May, 1937)

Through the kindness of Professor W. S. Patton and Dr. A. M. Evans, the writer is able to report on a small but extremely interesting collection of Ethiopian Simuliidae in the possession of the Department of Entomology of the Liverpool School of Tropical Medicine. In all, some seven species are represented, and of these one from Rhodesia proved to be new. Included in the collection are specimens of two species from Nyasaland collected in 1913 by Dr. H. S. Stannus (1913) and Mr. E. Ballard; these were determined by Roubaud* as *Simulium latipes* Meigen and *Simulium pusillum* Fries, and were labelled as varieties but remained unnamed. Further examination, however, proved them to be no other than *Simulium nigratarsis* Coquillett and *Simulium hargreavesi* Gibbins, which are noted for the first time from Nyasaland. Unpublished records of *Simulium damnosum* Theobald, *S. adersi* Pomeroy, *S. alcocki* Pom. and *S. unicornutum* Pom. are also given in the present communication.

Simulium arnoldi sp. nov.

Described from 4 males, one of which, with its dissected terminalia, is mounted on a slide. The species somewhat resembles *Simulium ruficorne* Macquart in the pattern on the thorax (fig. 1), but is strikingly different in the

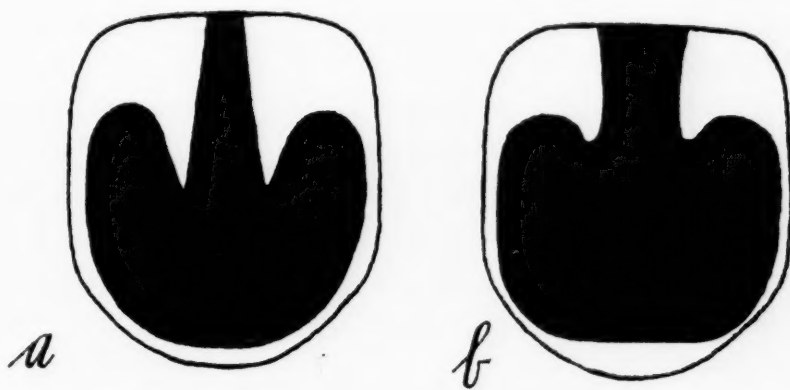


FIG. 1. Thorax of male, drawn to show the pattern on the mesonotum. *a.*—*Simulium arnoldi* sp. nov.; *b.*—*Simulium ruficorne* Macquart.

*See Editor's note, *Review of Applied Entomology*, Series B, 2, 35 (1914).

terminalia (Gibbins, 1936). The female, larva and pupa are unknown. The species is named in honour of its discoverer, Dr. G. Arnold.

MALE. Length 2 mm. ; wing 2 mm.

Head. Front and clypeus light grey, the latter with sparse pale hairs. *Antenna* dark brown, covered with the usual fine ash-grey pubescence, scape, first and base of second segment light brown. *Palp* dark brown, covered with a fine pale pubescence, sensory spot small.

Thorax. *Mesonotum* slate-grey, sparsely covered with pale scales and with a striking velvet-black pattern (fig. 1, *a*) sparsely covered with deep golden scales, the pattern consisting of a large area produced anteriorly into a long broad median stripe with converging sides, and with a broad rounded projection on either side, about half the length of the median stripe. *Scutellum* dark brown, with long outstanding pale hairs along the posterior margin. *Pleura* slate-blue, lacking the patch of soft hairs on the membrane behind the thoracic spiracle. *Halteres* pale yellow, with brown base. *Wing* normal, pale and lacking hairs on the subcosta and a basal cell.

Abdomen velvet-black, sparsely covered with short golden scales and with a large slate-grey lateral patch covering all but the first four segments; first tergite dark brown, with a dense fringe of long yellow hairs. *Terminalia** (fig. 2). *Coxite* (fig. 2, *a*) in ventral view broad, with an outer projection, and about half as long again as the clasper. *Clasper* narrowing gradually to a rounded end, with a small hooked tooth at the tip (fig. 2, *b*). *Phallosome* (figs. 2, *c* ; 2, *d* ; 2, *e* ; 2, *f*) : *anterior part* long and narrow in lateral view, with a short distal projection on its posterior edge and with a delicate anterior process covered with fine setae; in ventral aspect (fig. 2, *d*) the anterior part assumes the shape of an inverted Y, and is expanded and rounded anteriorly; median process (fig. 2, *e*) consists of a heavily chitinized bar; *posterior part* membranous, with heavily chitinized parameres arranged in the form of a distal pad, armed with strong backwardly directed conical teeth. Apodeme long and narrow, with its basal half strongly chitinized. *Cercus* (fig. 2, *g*) small and lightly chitinized, with about 8 long spines.

Legs dark brown, with the exception of the femora and tibiae of the front and middle legs, apex of femora, basal third of tibiae, basal two-thirds of first tarsal segment and base of second tarsal segment of hind leg, which are golden; calcipala present and pedisulcus distinct.

HOLOTYPE male, Rhodesia, Victoria Falls, December, 1914, G. Arnold.

PARATYPES 3 males, same data.

***Simulium damnosum* Theobald**

New records of caught females agreeing with specimens collected from the type locality at Jinja, Uganda, are as under.

*For terminology used, see Gibbins (1935).

SIERRA LEONE. Numerous females: Masimbiri, Mabonto, March 12th, 1928; Magburaka, Seli River, March 5th, 1928; Batkanu, Karene District, February 5th, 1928, D. B. Blacklock; Massehloo, near Mabang, R. M. Gordon. Single female: Gbaima, Anglo-Liberian Frontier, S.E. Murphy.

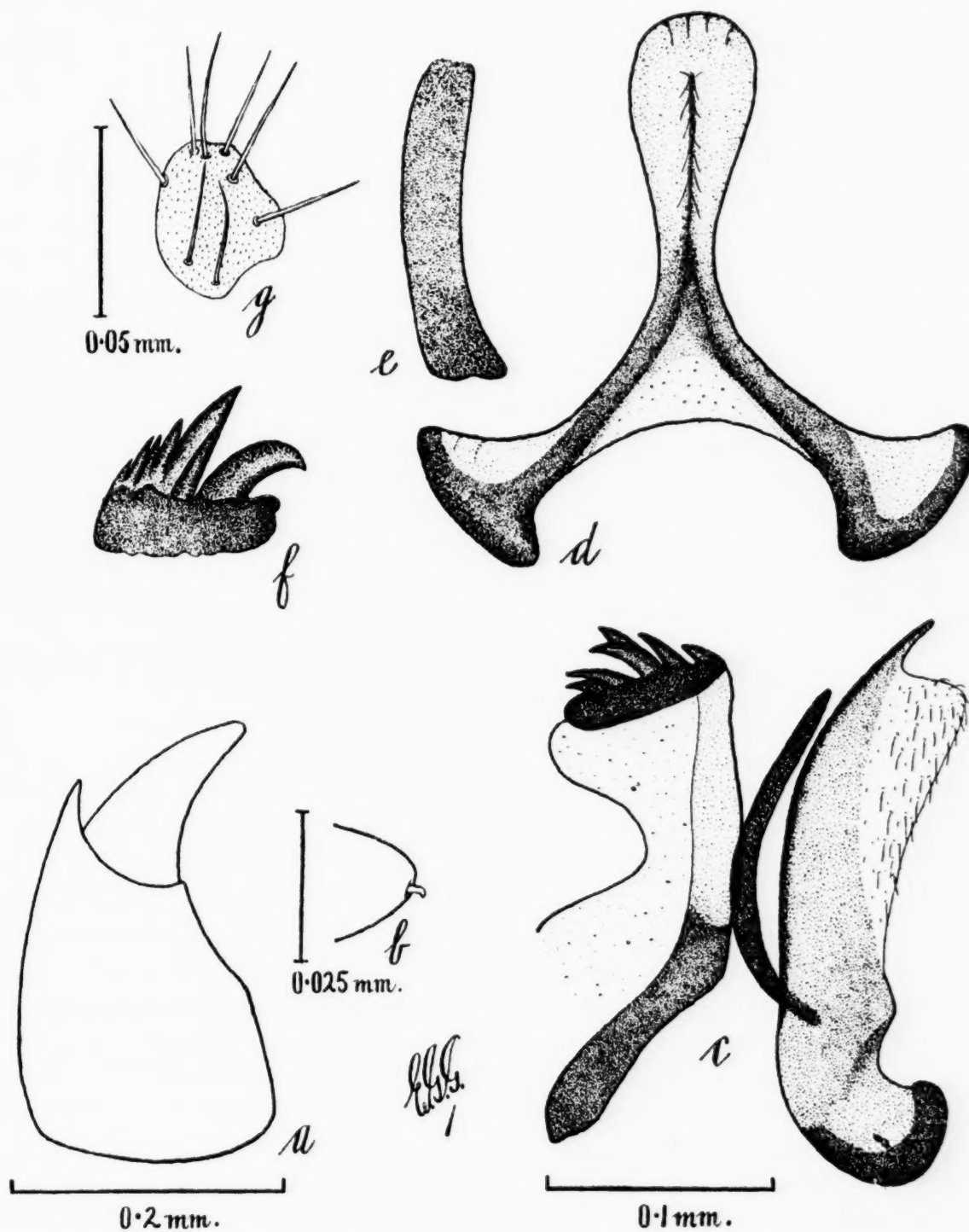


FIG. 2. *S. arnoldi* sp. nov. Terminalia of male. *a*.—Coxite and clasper; *b*.—Tip of clasper enlarged to show hooked tooth; *c*.—Lateral view of phallosome; *d*.—Anterior part of phallosome in ventral aspect; *e*.—Median process in ventral view; *f*.—Paramere in ventral view; *g*.—Cercus. (Magnification of *d* and *e* as in *c*; of *f* as in *g*.)

BELGIAN CONGO. Three females: François Joseph Falls, River Kwango, 1936, J. Schwetz.

***Simulium adersi* Pomeroy**

Previously noted from Nsadzi and Nkosi Islands. It is of interest to record the presence of this species on another island in Lake Victoria.

UGANDA. Single female: Lake Victoria, Damba Island, H. L. Duke.

***Simulium alcocki* Pomeroy**

SIERRA LEONE. Pupae, Jiamia Nimi Koro and Tumbudu, March, 1930, E. P. Hicks.

***Simulium hargreavesi* Gibbins**

One male and two females, which had been bred from pupae obtained in Nyasaland and labelled *Simulium pusillum* Fries (var.), have been found to belong to this species. The Simuliid has hitherto been known only to occur in Uganda.

NYASALAND. One male and two females bred from pupae, Zomba, February, 1913, H. S. Stannus.

***Simulium nigratarsis* Coquillett**

A single male labelled *Simulium latipes* Meig. (var.) proved to belong to this species.

NYASALAND. One male, Zomba, Mponda, January 1st, 1913, E. Ballard.

***Simulium unicornutum* Pomeroy**

A widely distributed species in tropical Africa, occurring in very slowly flowing streams.

SIERRA LEONE. Mature larva, March, 1930, E. P. Hicks.

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THE BRITISH SPECIES OF THE SUBFAMILY SARCOPHAGINAE, WITH ILLUSTRATIONS OF THE MALE AND FEMALE TERMINALIA

(Continued from Vol. 30, page 350)

BY

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AND

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(Received for publication 13 May, 1937)

Sarcophaga filia Rondani. DIAGNOSTIC CHARACTERS OF ADULTS VISIBLE EXTERNALLY. ♂. This is one of those species which are easily recognized when once known. The shape of the abdomen is characteristic: it is somewhat cylindrical, the third visible tergum is widened below posteriorly, the fourth is markedly larger than either of the preceding, being longer and much widened below, the result being to give a clubbed appearance to the end. The sterna seem smaller than usual in proportion, partly because they are placed nearer to the base of the abdomen, the first and second being largely overhung by the base of the hind legs, and the third and fourth extending but a short distance, if any, past the middle. All other species with which it might have to be compared have a more or less flattened abdomen, the fourth tergum no larger above or below than the third, and the sterna placed much farther back. The general colour effect is yellowish, owing to its golden tomentum, the other species concerned being of the usual tone, with silvery tomentum.

In the key in 'British Tachinidae' (Wainwright, 1928) it runs down, on account of its black terminalia (black, at least, in all British specimens seen), 3 D.C., long strong marginals on second abdominal segment, and bare first longitudinal vein, to a small group, the others of which are *agnata* Rond., *crassimargo* Pand. and *rosellei* Böttch., though it might be run to couplet 25, as there are usually some irregular, longish, almost bristle-like, acrostichal hairs. It can be distinguished from *clathrata* Mg., *crassimargo* Pand., *agnata* Rond. and *rosellei* Böttch. by the greater width of the frons, which even in this sex is quite four-fifths of eye-width at vertex, as seen from above, whilst in none of the others is it over three-fifths; from *nigriventris* Mg. and *villeneuvei* Böttch., an easy distinction lies in the presence of strong brush-like clusters of short stiff setae on the hind trochanters of *filia*, of which there is no trace in the other two species.

It is a medium-sized species, usually about 7-8 mm. long. The antennae are short, third joint about one-third longer than the second; arista with rather long ciliation; genae wide (eyes being small), fully twice as wide as the third

antennal joint, with numerous hairs, of which about three of the lower ones are long and strong; epistoma produced; orbits comparatively wide; frontalia about half as wide again as one orbit; outer verticals developed. Thorax shows three well-marked stripes, the centre one extending to the scutellum; 3 postsutural D.C., one pair of small prescutellar acrs.; no presutural intra-alar. Scutellum with well-developed apicals; basals and preapicals strong and long; discals small. Abdomen with long strong pair of marginals on second tergum; the usual shifting tessellation well developed; the two genital segments (terga 7 and 10) large and all shining black, with a row of strong bristles on hind margin of first (7) and long black hairs over all. Sterna moderately short-haired, longer than wide; lamellae of apical one (5) having a well-developed setal brush, but no inner marginal fringe. Wings with long costal thorn; third costal section just about equal to the fifth; first vein bare; usual setae on third.

LEG CHAETOTAXY. Fore femora with usual postero-ventral series of setae only moderately long and strong; some long fine hairs at base, rest of posterior pilosity neither long nor dense; postero-dorsal series in a double irregular row. Mid femora with antero-ventral series with about 4-6 stoutish bristle-like hairs on central portion, and a sparse row of short fine hairs at apex scarcely suggesting a comb; postero-ventral series complete with rather numerous long fine hairs, scarcely bristles, and those on apical portion longer than usual, irregular and not comb-like. Hind femora with a complete row of long fine hairs both antero- and postero-ventrally, including one long strong seta near apex antero-ventrally, and a few longer and stronger hairs on basal portion postero-ventrally. Hind tibiae with a postero-ventral fringe of long fine hairs beginning at about one-quarter from base and extending to apex; and a few odd hairs only antero-ventrally.

♀. Again *filia* is one of the species probably easy to recognize even in this sex once the characters are appreciated. It is like the ♂ in most respects, with a similar cylindrical abdomen with much golden tomentum, so that it looks yellowish in contrast with the greyness of most species. It runs down in the key to the same group of species, but cannot so readily be separated from most of them by the greater width of the frons which, although wider than in all the other species, is perhaps not sufficiently so for the difference to be useful. It is over eye-width, about one-fifth wider, and in *agnata* Rond. and *clathrata* Mg. it is clearly under eye-width; but in the other species of the group it is at least somewhat wider. The shape of the abdomen is, however, very distinctive, as in the other species concerned this is more or less flattened and oval, with the terminalia beneath, whilst in *filia*, as already mentioned, it is cylindrical and the terminalia show posteriorly. The best character, however, lies in the shape, etc., of the sterna. The fifth is hidden; the sixth, which is partly seen between the edges of tergum 6, is for the most part smooth, bare and shining, with the posterior and lateral margins, as far as seen, raised and rugose, and with a row of about six bristles on the posterior margin. The seventh is placed at an angle

to the sixth, faces postero-ventrally and is fully seen; its anterior portion is a half-moon-shaped depression, shining and bare, and is bordered posteriorly and laterally like the sixth, with a raised rugose margin with some short hairs but no marginal series of long setae. The sixth tergum is prominent, projecting well beyond the edge of the fifth, and is complete, with no division or notch at top; it is covered with yellow tomentum; below the posterior edge of this tergum, and between it and the anal cerci, is a conspicuous smooth recess.

The ♀ agrees with the ♂ except for the usual sexual differences; the apical scutellars fail; but the marginals on the second abdominal are long and strong. It is perhaps even yellower than the ♂. There is the usual hind marginal series of strong upright setae on the fifth tergum, with only a few very short ones of the downward curved series. The sixth tergum has a row of strong hind marginal setae, which fails for a short space at the top; the hind margin is almost perpendicular. Sterna 2 and 4 each bear a pair of strong setae on front margin.

LEG CHAETOTAXY. Front femora as in ♂. Mid femora with antero-ventral series consisting only of two strong setae about middle and a sparse row of short hairs on apical part; postero-ventral series with about four long strong bristle-like hairs on basal half, and apical half with a row of somewhat irregular short (but longer than in antero-ventral series) hairs, no comb. Hind femora without the series of long hairs of the ♂, with only three widely separated long setae on antero-ventral aspect, and three or four similar ones on basal two-thirds of postero-ventral aspect. No fringes.

NOTES. *Filia* is not a common British species, and seems to be southerly in its distribution. We possess odd specimens from Folkestone, Wye Downs, Selsley (Gloucestershire), Tenby, Wicken Fen and Princes Risboro, and we have seen others, mainly from southern seaside localities, the most northern being Devil's Ditch, Newmarket (J. E. Collin), and Yarnton, Oxford (J. Collins). According to Böttcher, specimens occur with more or less red about the terminal segments; but he says that these are chiefly found in the southern part of its area, which he quotes as being mid- and south Europe and East Prussia. Lundbeck does not include it in his Danish fauna.

DIAGNOSTIC CHARACTERS OF TERMINALIA. ♂. Fig. 1. Each anal cercus is a long plate, closely united to its fellow for more than three-quarters of its length. In profile (fig. 1, *d*) it is seen to be characteristically expanded at the end, terminating by bending upwards in a short beak-like point. The ventral view of the two cerci are illustrated in fig. 1, *c*. The phallosome (fig. 1, *a*) is long, the distal part expanded and ending in a long wide median process dorsally, which is membranous; the proximal part of the phallosome is a long chitinous tube, convex ventrally, and hinged ventrally to the distal chitinous part and dorsally by the usual membrane, which is long, folded and ribbed; on the ventral side of the distal part there are a pair of wide chitinous processes directed towards each other, their strong tooth-like pointed ends almost meeting

(fig. 1, *e*); distal to them is a short median bent process. The posterior paramere (fig. 1, *a, f*) is an upstanding plate which ends in an abrupt, sharp, bent, beak-like point; it has two prominent hairs on the dorsal surface near the distal end, one very long; the posterior paramere (fig. 1, *a*) is a long, broad, bent plate terminating in a blunt point; it has a row of four or five long hairs (one very

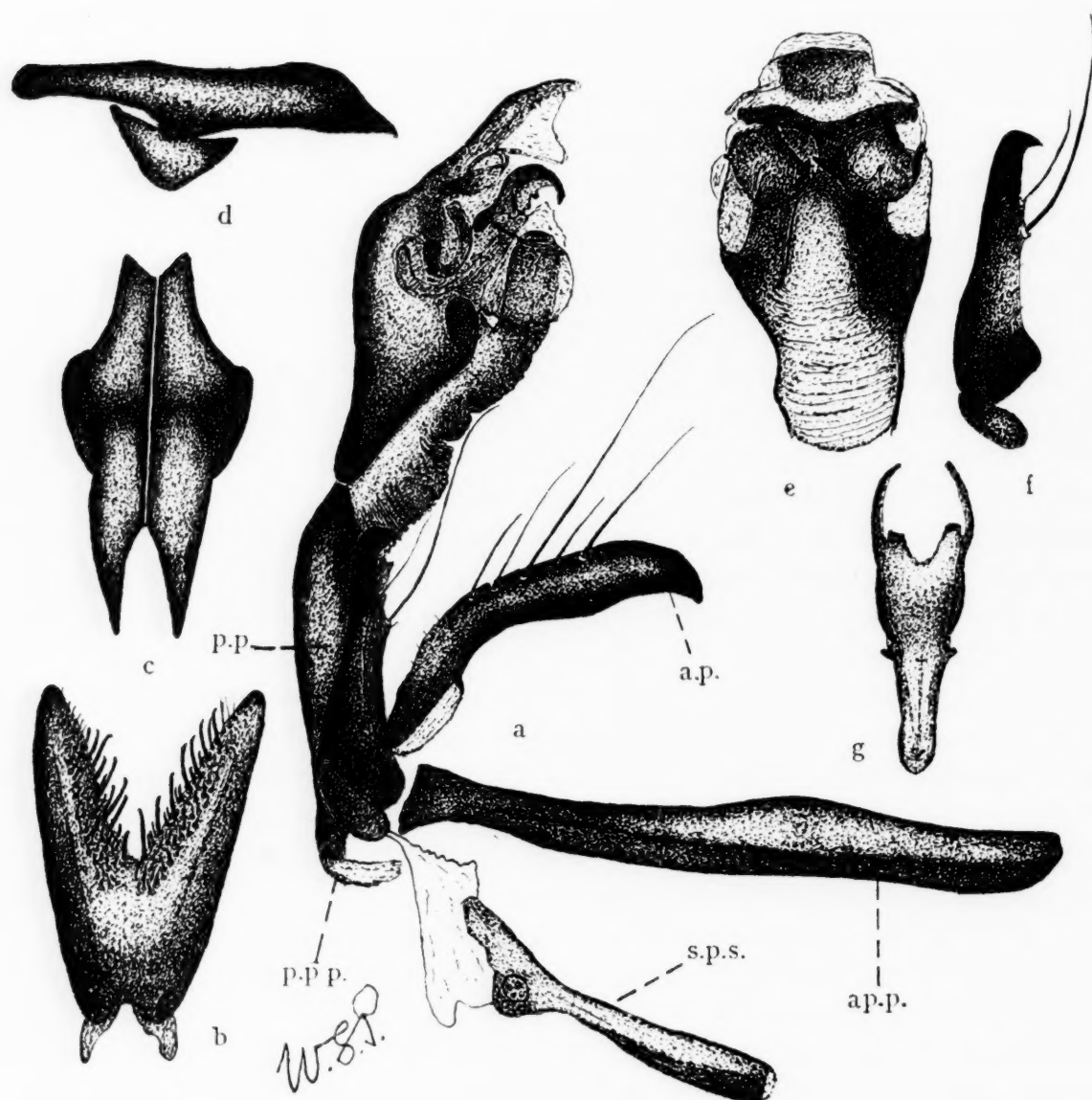


FIG. 1. *a*.—Phallosome and one paramere of *filia* in side view; *ap.p.*—Apodeme of phallosome; *p.p.*—Posterior part of paramere; *p.p.p.*—Posterior process of phallosome; *s.p.s.*—Sperm pump sclerite; *b*.—Fifth sternum; *c*.—Ventral view of anal cerci and distal segments of ninth coxites; *d*.—Lateral view of anal cercus and distal segment of ninth coxite; *e*.—Dorsal view of end of phallosome; *f*.—Posterior part of right paramere; *g*.—Ninth tergo-sternum.

long) along its ventral border, and a row of four or five minute hairs near the basal end. The apodeme of the phallosome (fig. 1, *a*) is very long. The ninth tergo-sternum (fig. 1, *g*) is narrow and long, with two raised parts on the dorsal surface, on which the anterior parameres ride. Sternum 5 (fig. 1, *b*) is long, the

stem short and broad, with a deep narrow emargination and two processes; the arms are long and wide, with numerous stiff hairs bent at their ends.

♀. Fig. 2. Tergum 6 (fig. 2, *c*) is complete, as already noted; tergum 7 is wanting. Sternum 5 (fig. 2, *a, b, f*) is long, narrowing posteriorly to a point; from the illustrations it will be noted that the shape of the posterior end varies somewhat. Sternum 6 (fig. 2, *a, b, f*) is also long and is wider, the posterior end rounded; in cleared specimens there is a very distinct arched line about the middle, which though it varies in size is very constant. Sternum 7 (fig. 1,

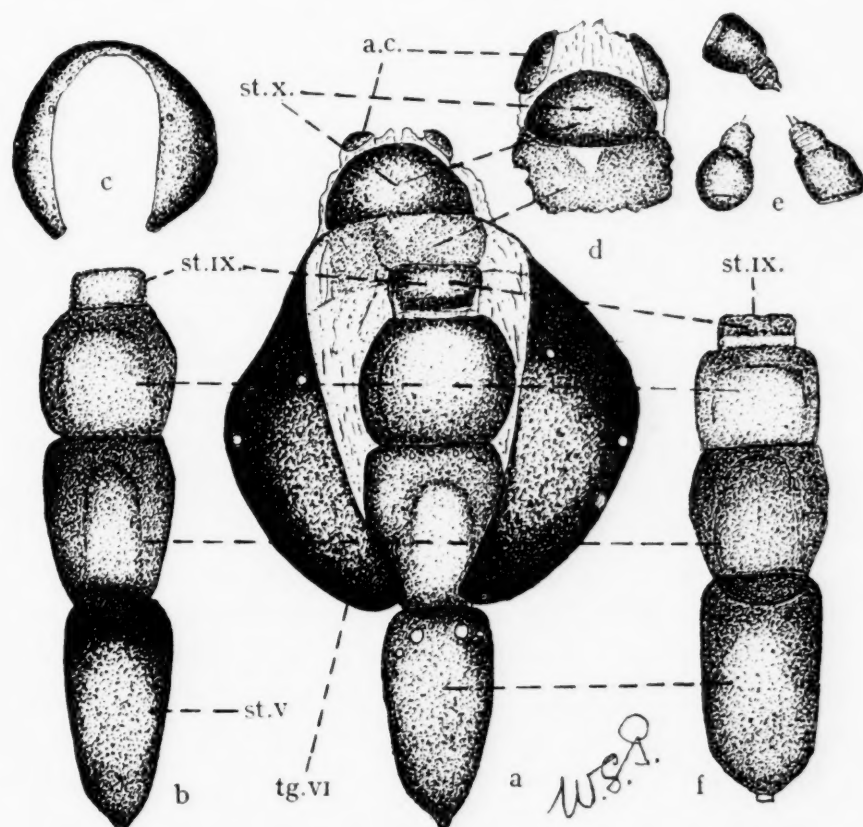


FIG. 2. *a*.—Ventral view of terminalia of ♀ *filia*, to show diagnostic characters; *a.c.*—Anal cerci; *st.v*, *st.ix*, *st.x*.—Fifth, ninth, and tenth sterna; *tg.vi.*—Sixth tergum; *b*.—Fifth, sixth, seventh and ninth sterna, showing variation in shape when dissected off and mounted flat without compression; *c*.—Sixth tergum, to show that it consists of a single plate; *d*.—Ventral view of end of terminalia, to show anal cerci, tenth sternum and attached accessory plate; *e*.—Spermathecae; *f*.—Fifth, sixth, seventh and ninth sterna, to show structural variation.

a, b, f) is a shorter wider plate; its shape, when still attached *in situ* (fig. 2, *a*), appears expanded laterally, but, when dissected off and mounted flat, it is not nearly so expanded and may be almost square; it also has a dark arched line in the middle which was present in the three specimens illustrated. Sternum 9 (fig. 2, *a, b, f*), which forms the outer ventral wall of the genital opening, is a small rectangular plate, and is very characteristic of this species. Sternum 10 (fig. 2, *a, d*) is a large rounded plate and has a large, somewhat irregularly shaped plate attached to its proximal end (fig. 2, *d*), which forms the dorsal wall of the genital opening. This plate, of course, cannot be seen except on dissection.

These characters of the sterna taken together are very characteristic of the species, rendering identification easy.

Sarcophaga haemorrhoidalis Meigen. DIAGNOSTIC CHARACTERS OF ADULTS VISIBLE EXTERNALLY. ♂. *Haemorrhoidalis* belongs to a group of large species all normally with 4 D.C., of which, however, only the two hinder ones are well developed, the others being small and even inconspicuous, all the presutural macrochaetae being similarly reduced. In all these species the marginals fail on the second visible abdominal segment, there are no presutural acrostichals, and no setae on the first long vein, and in general the chaetotaxy tends throughout to slight development. The group contains some species with the genital segments all black, others with them more or less so, and in still other species individuals vary, and may be either black or red. *Haemorrhoidalis* is one of those whose second genital segment (tergum 10) is always red, and usually part of the first segment also, and *falculata* Pand. (which see) is another. *Tuberosa* Pand. and *albiceps* Mg., both of which have recently been found in this country, are amongst the variable ones; so far, only the red-tailed var. *exuberans* Pand. of *tuberosa* has occurred here, and only the all-black form of *albiceps*, and, as the red variety of *albiceps* seems to be a southern form, it seems likely that all British ones will be black; nevertheless, the possibility of a red-tailed fly proving to be *albiceps* should always be borne in mind. The ♂♂ are, of course, readily distinguished if their terminalia are examined, but external differences are slight; *haemorrhoidalis*, however, may be recognized by the entire absence of the usual pair of prescutellar acrostichals, present in the other three species mentioned; and by the presence of two or more long strong macrochaetae on the hind margin of the first genital segment, of which there is no trace in the others.

Haemorrhoidalis is a large species, 12 to 15 mm. long, with yellowish tomentum. The frons is wide, about three-quarters of eye-width as seen at vertex; genae wide; head covered with yellowish tomentum; lateral verticals missing or weak; genae with numerous, short, hair-like bristles; facialia with a few very short setae, reaching fully to eye middle; antennae long, third joint two and a half times as long as second; arista long, plumose; palpi rufous, dark-tipped. Thorax, see above for chaetotaxy; three well-marked, equally broad, complete stripes, narrower than interspaces. Scutellum with well-developed crossed apicals; two other pairs of long strong marginals, and a pair of well-developed discals. Abdomen, chaetotaxy as above; well covered with yellowish tomentum, with the usual tessellated markings; sterna 3 and 4 nearly bare; sternum 5 deeply cleft, with fringes of strong setae on inner side, not, however, forming a definite 'brush.' Wings with no costal thorn; third costal section half as long again as the fifth.

LEG CHAETOTAXY. Front femora with usual postero-ventral row, a little weak and irregular; the posterior pubescence short and not greatly developed. Mid femora with antero-ventral row consisting of short, moderately thick setae,

all about the same length, about eight scattered on basal three-fourths, and then an equal number closer together on apical quarter, forming an indefinite comb; postero-ventral aspect with dense long pubescence in place of usual bristles on basal two-thirds, ending abruptly, and end third with well-developed comb (Enderlein's ctenidium). Hind femora with a row of scattered short bristles on antero-ventral aspect, and no postero-ventral series. Hind tibiae with well-developed 'fringes' of hairs on postero-ventral and antero-ventral aspects, the postero-ventral being longest and most complete. Hind coxae with a cluster of short thick setae, on basal portion only, forming a small brush.

♀. The ♀♀ of this group are hard to distinguish from one another, lacking the assistance of the ♂ terminalic characters; and the variation in coloration is more confusing in this sex, as many other species, notably *carnaria* L., are occasionally more or less red about the edges of tergum 6, and the external characters of the ♀♀ of all the species of this group are generally very alike. *Haemorrhoidalis*, however, can perhaps usually be separated from *falculata* and *tuberosa* by the total failure of the usual pair of prescutellar acrostichals, which is present in both those species. In *albiceps* these bristles are never very large and may be indistinguishable in the ♀; but it is probable that British specimens will prove normally to be black-tailed, and so not be liable to confusion with *haemorrhoidalis*. Probably the easiest character by which to distinguish *haemorrhoidalis* ♀ from all the rest of this group, so far at least as our British species are concerned, will lie in the form of tergum 6. This is brilliantly rufous and shining, and projects considerably beyond the edge of sternum 5; it is divided above, and the two sides of the slit are not parallel, but curve outwards behind, leaving a V-shaped notch with rounded corners. There is a row of setae along nearly the whole hind margin right up to the notch, stronger in the middle of each side and finer above and below. The sterna are also characteristic. The third, fourth and fifth are squarish, but comparatively narrow, each with a few short setae on hind margins; sternum 6 is very nearly double the width of sternum 5, and nearly twice as wide as long, rufous posteriorly, with about six or eight strong hind marginal bristles; sternum 7 is rather longer, not quite so wide, and all rufous, with a somewhat half-moon-shaped, shining, bare convexity occupying most of the segment and standing out conspicuously; there are two bristles in each corner behind. The hind margin of tergum 5 bears only a few short bristles in the decumbent series.

Frons at vertex a trifle wider than an eye; apicals failing on scutellum; otherwise most of the outer characters as in the ♂.

LEG CHAETOTAXY. Fore femora with usual postero-ventral series fully developed, no special pilosity; mid femora with three or four short stout bristles on basal half, and a row of short fine ones, scarcely suggesting 'combs,' on apical half on both postero-ventral and antero-ventral aspects, usually one or two more in posterior than in ventral series; hind femora also with three or four scattered irregular strong bristles on each aspect.

NOTES. *Haemorrhoidalis* is rare in the British Isles. Like the other three species with which it has been compared (*tuberosa* Pand., *falculata* Pand. and *albiceps* Mg.), it is a very widely distributed species, but seems more at home in warmer climes than in ours. Lundbeck says that it is found in 'all Europe, most of Asia and Africa and also North America,' but he adds that it is rare in Denmark, as he has only two ♂♂, so that probably we are at the northern limit of its distribution. We possess two British specimens only, both ♀♀,

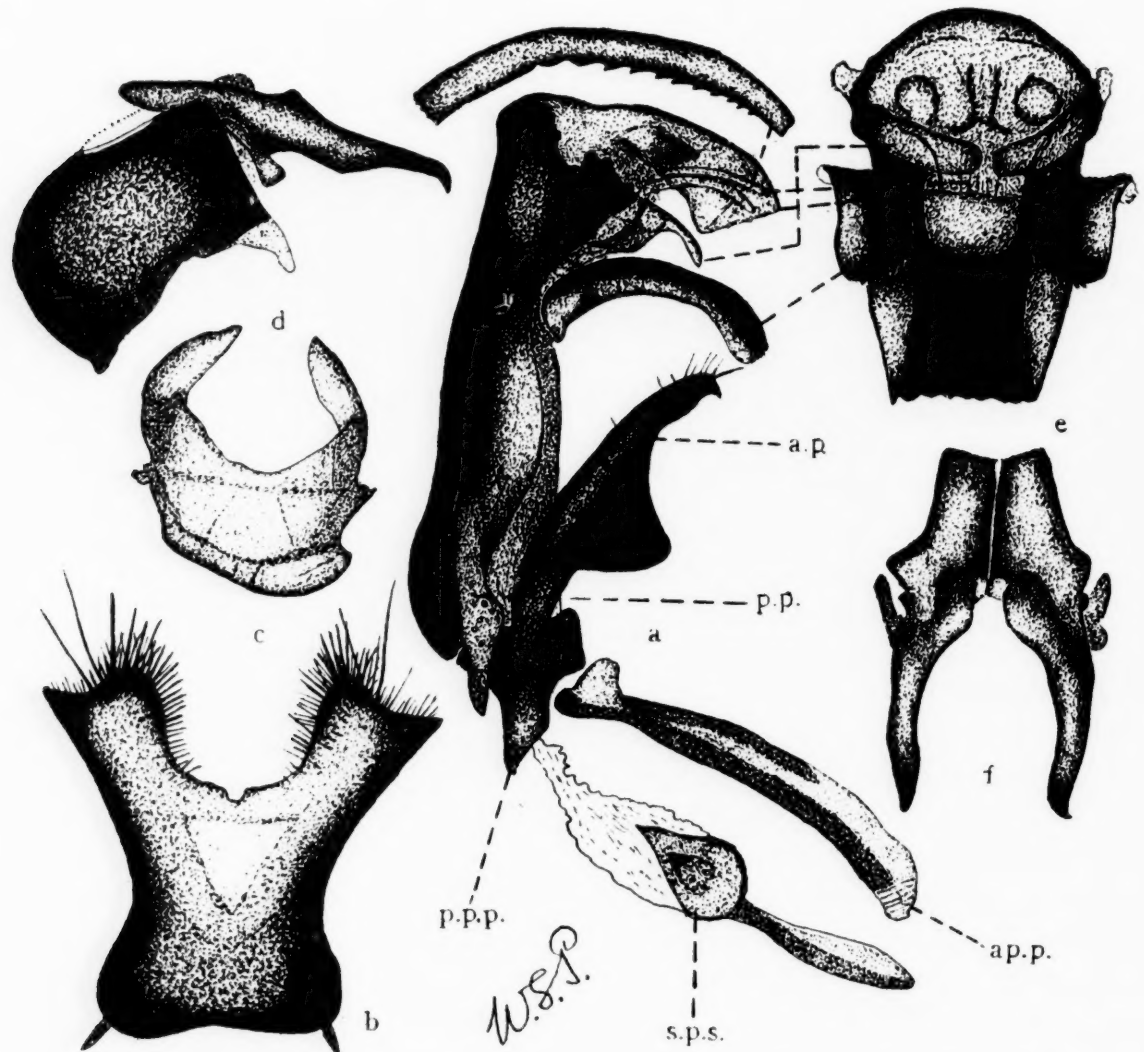


FIG. 3. *a*.—Phallosome and one paramere of *haemorrhoidalis* in side view; lettering as in fig. 1, *a*; *b*.—Fifth sternum; *c*.—Ninth tergo-sternum; *d*.—Tenth tergum, anal cercus and distal segment of ninth coxite in side view; *e*.—Dorsal view of end of phallosome; *f*.—Ventral view of anal cerci and distal segments of ninth coxites.

taken by Mr. H. W. Andrews at Eltham; but we have also seen or heard of odd specimens from Blundellsands, South Lancashire (Rev. L. W. Grensted), Plymouth district (Col. C. G. Nurse), Coombe, Warwickshire (J. W. Saunt), Colchester (B. S. Harwood), Cambridge (two ♂♂, F. Jenkinson), Crumbles, Eastbourne (H. W. Andrews), Dorchester (Dr. C. D. Day), and Oxford (A. H. Hamm)

DIAGNOSTIC CHARACTERS OF TERMINALIA. ♂. When seen in side view, each anal cercus is a long, rather narrow plate, with a characteristic hump about the middle on the ventral side; another raised area is also visible behind it, but it should be noted that it is in the middle line, at the point where the two cerci join each other; the distal free end narrows and bends up as a strong pointed beak (fig. 3, *d*). In ventral view (fig. 3, *f*) it will be noted that about three-quarters of each cercus is free, the remainder being fused with its fellow. The distal segment of the ninth coxite (fig. 3, *d*) is long and narrow, the free end slightly dilated. The phallosome (fig. 3, *a*) is long and of a very characteristic structure: the proximal part is very short, with a short pointed (in side view) posterior process, which is actually broad when seen from the ventral view, as in most species; the distal segment of the phallosome is long and strongly chitinized, and ends in a broad, membranous, hood-like process, directed ventrally; a little distal from the middle on the ventral side there is on each side a long broad chitinous plate, which projects freely and is finely serrated along its rounded end; distal to it there is on each side a broad finger-like semi-chitinous plate directed towards the middle line, almost meeting its fellow (fig. 3, *e*); under the hood-like end there is a pair of long serrated chitinous rods, one of which is shown enlarged in fig. 3, *a*. No British species has a phallosome like this, and at present we know of only two other species (out of some 60 species now studied), one in South Africa and one in China, which have a similar phallosome. The posterior paramere (fig. 3, *a*) is a short plate, ending in a bent point with two hairs near the end; the anterior paramere (fig. 3, *a*) is a long plate expanded, as shown in the illustration, about the middle on the ventral side and ending in a short, sharp, beak-like point; there are many short hairs on the inner surface, especially near the end. Sternum 5 (fig. 3, *b*) is long and wide, the lobes short and extended into pointed processes externally, their rounded ends and inner margins armed with numerous short, rather stiff, hairs.

♀. Fig. 4. Tergum 6 (fig. 4, *a, e*), as already noted, is very characteristic, consisting of two distinct plates, their free dorsal ends sloping outwards and forming a notch. Tergum 7 is wanting. Sternum 5 (fig. 4, *a*) is rectangular in shape; sternum 6 (fig. 4, *a, d*) is a large wide convex plate, shaped as shown in the illustrations; sternum 7 is about as wide, but slightly longer, with rounded sides; sternum 9 (fig. 4, *a, d*) is a very narrow plate, attached to the distal end of sternum 7, the sides strongly chitinized and projecting like a rod at each side. Sternum 10 is a large triangular-shaped plate with numerous fine hairs; it has attached to its proximal side a large accessory plate (fig. 4, *c, ac.p*). The signum (fig. 4, *c*) is large and strongly chitinized. This combination of characters, especially the notched tergum 6, render identification easy.

***Sarcophaga falculata* Pandellé.** It seems well at this point to call attention to a fact overlooked when describing this species in Part 1, namely, that the ♀♀ can be recognized by externally visible features of the apical segments. Tergum

6 projects beyond sternum 5, but not so much as in the ♀ *haemorrhoidalis*, and is not divided or notched above, although there is a carina where the division would be; it is dull, too, not shining, with some tomentum. The red seventh sternum is very characteristic: on the middle of the anterior margin is a small shining convexity like a half bead; the hind margin also is raised and curved, so that the raised portion is shaped like a broad Cupid's bow; in each hollow in front is one strong black bristle. Sternum 6 is wholly black, large, but not so wide as in *haemorrhoidalis*, and has one long bristle in each hind corner. On the hind margin of tergum 5 are more and longer bristles in the decumbent series than in *albiceps*.

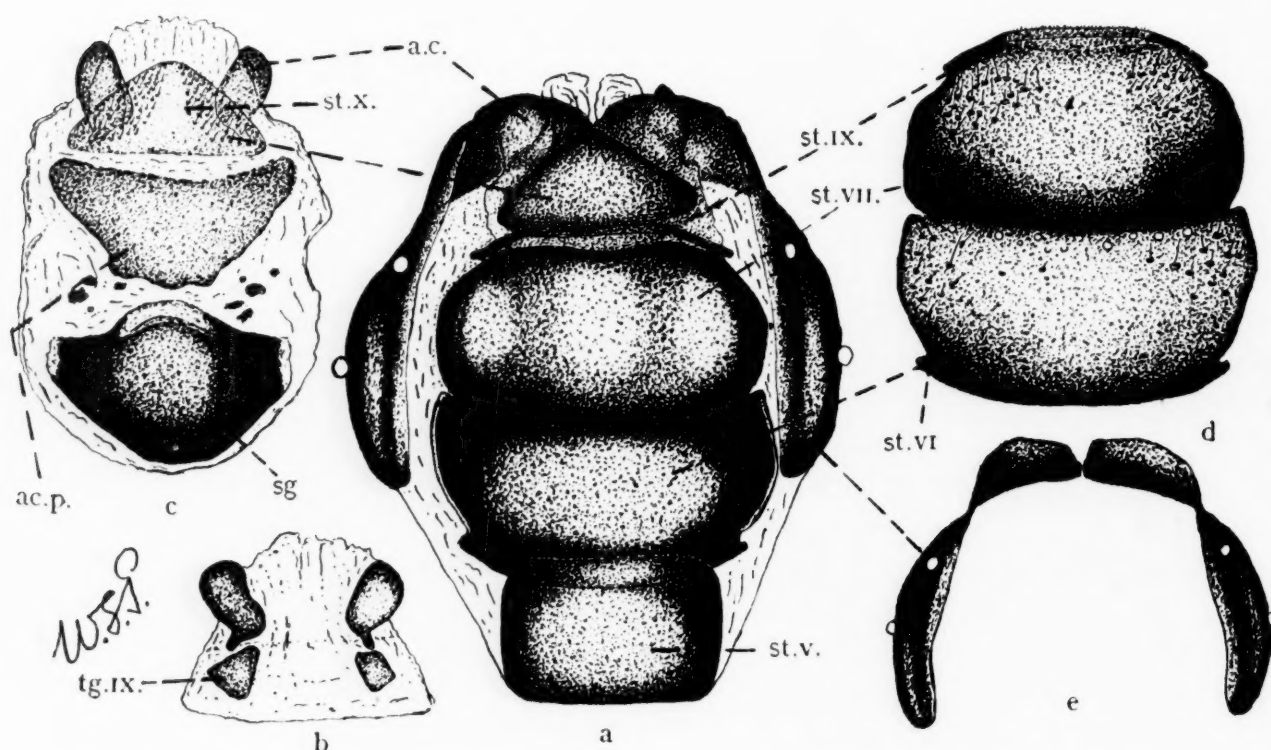


FIG. 4. *a*.—Ventral view of terminalia of ♀ *haemorrhoidalis*, to show diagnostic characters; lettering as in fig. 2, *a*; *b*.—Dorsal view of end of terminalia, to show anal cerci and tenth tergum in two parts; *c*.—Ventral view of end of terminalia, to show anal cerci, tenth sternum, accessory plate (*ac.p.*) and signum (*sg.*); *d*.—Sixth, seventh and ninth sterna; *e*.—Sixth tergum, to show that it consists of two plates; note dorsal notch.

***Sarcophaga incisilobata* Pandellé.** DIAGNOSTIC CHARACTERS OF ADULTS VISIBLE EXTERNALLY. ♂. In the key in 'British Tachinidae,' *incisilobata* can be run down fairly easily, except, perhaps, that an occasional specimen may have small hind marginal setae on the second tergum, which may cause confusion. Of those species in the key which approach nearest, *filia* Rond. and *melanura* Mg., apart from the wider frons, which alone should enable them to be distinguished, have entirely shining black terminal segments, with a row of strong setae on the hind margin of the first, whilst in *incisilobata* the first genital segment is dusted with grey tomentum and there are no hind marginal setae.

Incisilobata has well-defined 'combs' on the mid femora, i.e., a row of short stout bristles on apical portion of both antero-ventral and postero-ventral aspects; these are represented in *crassimargo* Pand. only by a few irregular, finer, longer, bristle-like hairs; also, *incisilobata* has a well-developed 'brush' on the hind trochanters, consisting of a dense mass of very short stout setae, whilst in *crassimargo* there are only some longish fine hairs, not suggesting a 'brush.' *Rosellei* Böttch. lacks the 'combs' on the mid femora, but has the brush on the hind trochanters. *Rosellei* is, moreover, a stouter and more robust-looking insect. *Agnata* Rond. is easily recognized by its much narrower frons, which is only about two-fifths eye-width as seen from above; it has the combs on the mid femora, but only long hairs on the hind trochanters. Since the key was published, two other species have been added to the British list, which also run to couplet 19, namely, *hirticrus* Pand. and *laciniata* Pand. *Hirticrus* has no trace of trochanter 'brush' or femoral 'comb,' the femora bear practically no bristles but much long pilosity, and there are unusually highly developed fringes of long hairs on the hind tibiae on both sides, and on the mid tibiae also. *Laciniata* is very like *incisilobata*: the antennae are longer; third joint more than twice as long as second, in *incisilobata* only about two-thirds as long again; the first genital segment (tergum 7) has strong marginal setae, and there are combs on mid femora, as in *incisilobata*, but no brush on hind trochanters.

Incisilobata is a medium-sized species, about 8–12 mm. long. Lateral verticals developed little or not at all; arista not long, plumose; genae nearly double as wide as the rather wide third antennal joint, with a few bristles only, about three strong and a few fine ones; thorax with longish hairs between the presutural series of D.C., but no strong bristles; prescutellars not always present; 3 D.C.; no presutural intra-alars. Scutellum with crossed apicals and usual preapicals, basals and discals. Abdomen with the usual tessellated pattern, changing with position but tending to form three lines of dentoid markings. First genital segment dull, with tomentum and only fine hairs on hind margin; second segment shining black. Sterna with recumbent hairs not very long; apical sternum (5th) with a 'brush' of short stout setae, and also a fringe of longer ones on hind inner edges. Wings with a short costal thorn; third costal section rather longer than fifth; no setae on first longitudinal vein; only the usual ones on the third.

LEG CHAETOTAXY. Front femora with usual postero-ventral and postero-dorsal series of strong bristles. Mid femora: antero-ventral series with several (four or five) strong longish setae on middle portion, and on apical portion a 'comb' of rather fine, not very short, hairs; postero-ventral series, an irregular row mostly of long fine hairs on basal and central parts, with a more definite comb-like row of shorter stouter setae at apex. Hind femora with a row of about seven or eight strong bristle-like hairs beginning from about one quarter from base and extending nearly to tip on antero-ventral aspect; postero-ventral series, a rather dense row of longish fine hairs, with a few longer and stronger

ones towards apex. Hind tibiae with a fringe of long hairs on postero-ventral aspect, beginning about one quarter from base and extending to tip, and a few similar ones, placed irregularly on antero-ventral aspect.

♀. This is one of the ♀♀ of which we do not as yet feel very sure, and which we cannot distinguish with certainty from its allies. In the key it should run like the ♂ to couplet 19, and should easily be known from *melanura* Mg., which is one of the most clearly characterized of the *Sarcophaga* ♀♀; but we know very little at present about this sex of *laciniata* Pand. and *hirticrus* Pand. (the two species new to the British list), or about *agnata* Rond. *Melanura*, apart from its wide frons, yellowish tone, etc., can be recognized at once by its unusual terminal opening: this faces postero-dorsally; the fifth tergum completely overlies the upper part of the sixth, and in the hollow of the fifth, and between its upper edge and the anal cerci, lies a large smooth concavity, such as we have seen in no other species. What we believe to be the ♀ of *laciniata* has a very small genital opening, not visible from above, with no such concavity. Tergum 5 completely overlies the upper part of the sixth, and one apparently sees the edges of the two terga, one immediately above the other, each edge rather thick and covered with fine golden pubescence, so as to show two distinct golden strips; this seems also to be a distinctive character. It is likely that *hirticrus* will be recognizable through absence of bristles on the under side of the femora, and that it will resemble the ♂ in a clothing of dense fine hairs in place of the usual setae. *Crassimargo* Pand., *agnata* Rond. and *rosellei* Böttch. should be recognized by the hind marginal setae on second segment, which occur normally in all three species and are not likely to be found on the ♀ of *incisilobata*, although sometimes present on the ♂♂.

Incisilobata has tergum 5 projecting beyond the sixth, but the sixth can be seen beneath the fifth, except just at the top, where it appears overlaid. There is a row of the usual stout upright setae on hind margin of tergum 5, and a similar number of thinner decumbent ones; tergum 6 is divided above; it has the usual crossed marginal setae extending from near the top to where the edge curves under. The fourth, fifth and sixth sterna in the specimens examined were almost wholly hidden by the overlapping edges of the corresponding terga; sternum 7 is partly seen as a smooth rounded end; the terminal opening is normally very small, with no special features. There seems to be a slight hollow above the cerci, but it is very small and inconspicuous. Frons seen from above at vertex about one-quarter as wide again as an eye. Scutellum with no apicals, but with usual discals, etc. Abdomen wide, rather flattened, and oval in shape.

LEG CHAETOTAXY. General distribution as in ♂, but many fewer setae; on mid femora about two strong ones in front and three behind, and the combs present but weak; hind femora with an irregular unequal row of about six antero-ventral bristles and only about three or four long ones behind.

NOTES. *Incisilobata* is one of our commoner species, occurring apparently

more or less everywhere. We have it from many localities in England and from Nethy Bridge and Culbin Sands in Scotland. Böttcher says that it is widely distributed but not usually common, and Lundbeck gives its distribution as 'all Europe.' We have had the opportunity of examining a pair taken *in cop.* by the late Colonel Yerbury at Lyndhurst (May 17th, 1894) and now in the British Museum; and, although we have not dissected them, we feel pretty sure about them and believe that they confirm our identification of the ♀.

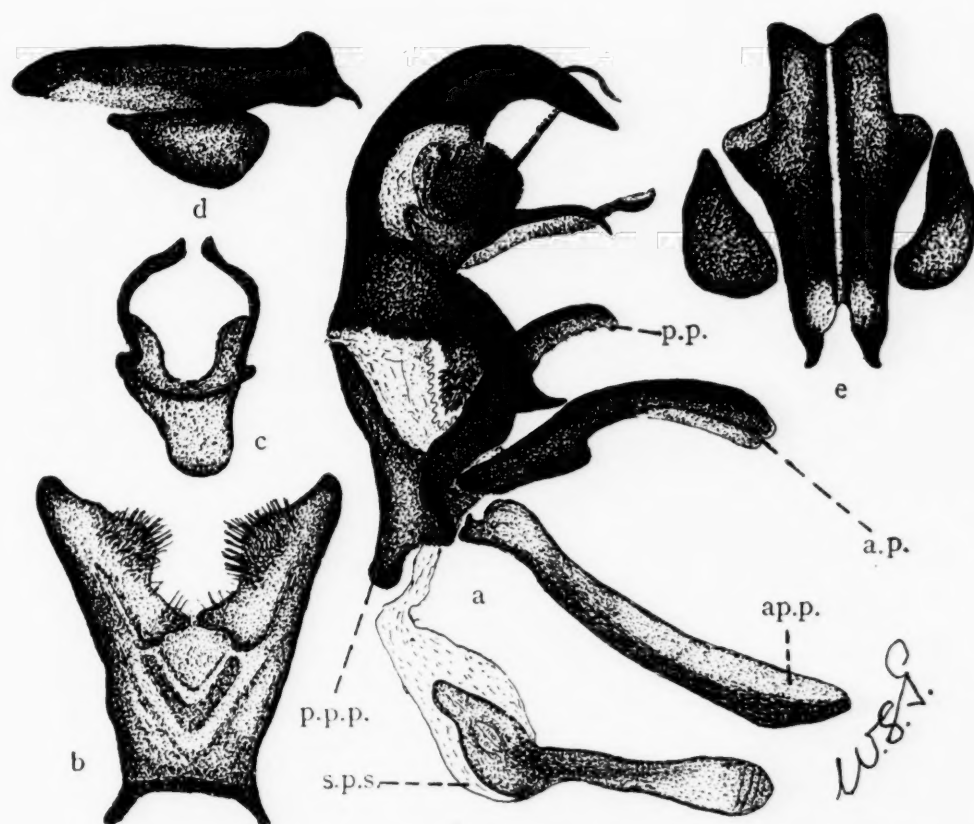


FIG. 5. *a.*—Phallosome and one paramere of *incisilobata* in side view; lettering as in fig. 1, *a*; *b.*—Fifth sternum; *c.*—Ninth tergo-sternum; *d.*—Anal cercus and distal segment of ninth coxite in side view; *e.*—Ventral view of anal cerci and distal segments of ninth coxites.

TERMINALIA. ♂. Fig. 5. Each anal cercus is a short plate attached to its fellow for almost its entire length, only the short raised end portion being free. In profile (fig. 5, *d*) it has a very characteristic appearance, the end raised ventrally into a round hump, terminating in a short pointed beak turned upwards; the distal segment of the ninth coxite is a wide rounded plate when seen in side view. The phallosome (fig. 5, *a*) is short, the distal portion ending in a stout, bent, pointed, chitinous process and a shorter narrower bent one; in addition, there are three much narrower chitinous processes arising from the ventral surface, one finely serrated and projecting towards the distal process, and two

others close together, one narrow and pointed, the other semi-chitinized, heavily spined and dilated at the end. The most characteristic part of the phallosome is a long process at the end of the proximal portion on the ventral side, shaped like a top-boot; it has a strongly spined membranous flap near the base. The basal part of the phallosome is joined as usual to the distal part by membrane on the ventral side and is long, the posterior process, as seen in profile, being short

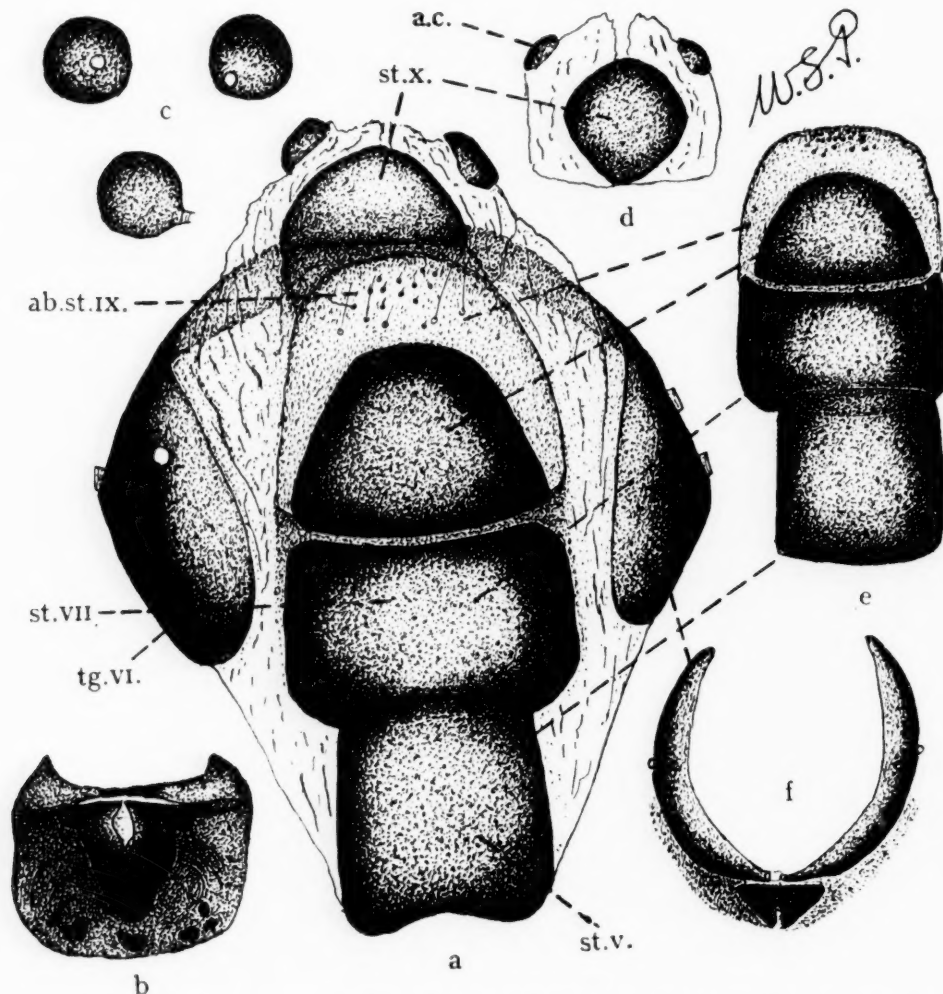


FIG. 6. *a*.—Ventral view of terminalia of ♀ *incisilobata*, to show diagnostic characters; lettering as in fig. 2, *a*; *b*.—Signum; *c*.—Spermathecae; *d*.—Dorsal view of end of terminalia, to show anal cerci and tenth tergum; *e*.—Sixth, seventh and ninth sterna; *f*.—Sixth tergum, to show that it consists of two plates; note triangular-shaped chitinized area in intersegmental membrane.

and blunt. The anterior paramere (fig. 5, *a*) is a long bent plate expanded towards the end; the posterior part (fig. 5, *a*) is a short bent plate. The ninth tergo-sternum (fig. 5, *c*) is relatively small, with long, narrow, posterior arms. The fifth sternum (fig. 5, *b*) is large, the lateral arms short, expanded on the inner side, and bearing a number of short stiff hairs.

♀. Fig. 6. Tergum 6 (fig. 6, *f*) consists of two distinct plates closely joined dorsally, and in the intersegmental membrane between tergum 5 and

tergum 6 on the dorsal side there is a chitinized area, which is most commonly triangular-shaped (fig. 6, *f*) but may be more irregular in shape. Tergum 7 is wanting. Sternum 5 (fig. 6, *a*) is a large rectangular plate, slightly overlapping (as is usual in the ♀♀ of the genus) sternum 6, which is a much wider plate and is wider than long (fig. 6, *a, e*) ; sternum 7 (fig. 6, *a, e*) is a narrower rounded plate, and has attached all round it a semi-chitinized plate, with many fine scattered hairs and usually a group in the middle of the distal end ; this semi-chitinous plate is in the position of sternum 9. Sternum 10 (fig. 6, *a, d*) is triangular-shaped. The signum (fig. 6, *d*) is well developed and is strongly chitinized in the middle in the shape of a heart.

(To be continued)

A STUDY OF *TRYPANOSOMA RHODESIENSE* RELAPSE STRAINS IN VITRO*

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(*Received for publication 1 June, 1937*)

INTRODUCTION

Liability of trypanosomes to immunological change during infection. Franke (1905) was the first to point out that pathogenic trypanosomes are liable to undergo a profound immunological change in an infected animal. Only in this way could he explain that the parasites in the blood during a chronic infection are insensitive to antibodies which have developed against the originally infecting strain. It has since become clearly established by many other workers that this fundamental alteration characteristically distinguishes the organisms of a relapse from those of the initial stages of an infection. The method of Ehrlich (1909), working with Roehl and Gulbransen, for demonstrating the altered nature of relapse trypanosomes, was to show that an infected mouse cured by drug treatment is immune, for a period, against reinfection by the original strain, but is fully susceptible to the parasites derived from a relapse in another mouse which had been infected with that same strain. Conversely, if a mouse be inoculated with relapse parasites and cured, an immunity follows against the relapse strain but not against the parent trypanosomes.

Stability of relapse variants. It was stated by Ehrlich (1911) that the parent and the relapse strains retain their separate character for many years, on passage through mice. The rapidly fatal form of infection normally occurring in mice is of service in this connection, because the steady multiplication of organisms, unopposed by any appreciable antibody response, ensures that the serological type of a trypanosome strain remains unchanged for an indefinite number of passages. Nevertheless, Mesnil and Brimont (1909), Neumann (1911), Braun and Teichmann (1912), Rosenthal (1913) and Ritz (1914) described cases where

* This work was assisted by a grant from the Chemotherapy Committee of the Medical Research Council, and the writers are indebted to Professor Warrington Yorke, F.R.S., for criticism and advice.

relapse strains, in the course of about 20–60 mouse passages, gradually resumed the parent strain character. Rosenthal and Ritz, however, each gave experimental evidence for the explanation that such strains arise in the first place as a mixture of organisms of the relapse and parent type, the latter gradually overgrowing and finally displacing the former, which, in conformity with Ehrlich's conception, need not then be considered as other than fixed mutants.

Multiplicity of relapse strain types from a single stem. Using Ehrlich's method described above, i.e., immunizing a mouse against one strain and then trying to reinfect with another, Neumann (1911) showed that 2 relapse strains obtained from the same stem under exactly similar conditions are not necessarily identical immunologically, and Ehrlich (1911) stated that he was able to recognize at least 5 such distinct variants. Using the same method, Ritz (1914) developed this line of enquiry further by isolating and testing against one another the organisms from successive relapses of a single mouse infection, which was given a sub-curative dose of drug as each relapse appeared. In two such experiments he demonstrated respectively 9 and 17 immunologically distinct relapse types. Sometimes the same variant recurred in a later relapse of the same infection or was duplicated in the other infection. Altogether Ritz disclosed 22 different types, and he believed that he was far from having reached the full number to which the original strain might give rise. In a later communication Ritz (1916) made somewhat similar observations for trypanosome infections of rabbits. Mice were inoculated daily from an infected rabbit, and the various strains thus obtained were tested against one another by Ehrlich's method. In this way it was shown that an apparently inexhaustible succession of immunologically different variants may appear in the same infection. This work is of further special value since the rabbit with which Ritz worked had been infected with a 'pure line' of *T. brucei*, from a single trypanosome.

With regard to the trypanosomes from the *first* relapses in different infections with the same strain, Ehrlich (1911) apparently thought that these were generally all of the same immunological type. Leupold (1928), relying mainly on the Rieckenberg reaction for differentiating her strains, concluded similarly that, whatever might be the position shown by Ritz for the trypanosomes of *successive* relapses of a chronic infection, in the case of *first* relapses of different mouse infections the organisms show only a limited variability. Thus, from the first relapses of over 50 mouse infections with *T. brucei*, 96 per cent. of the variants conformed to one, or other, or both of two main types. Raffel (1934), also using the Rieckenberg reaction, concluded that the first relapse strains of the same original strain of *T. equiperdum* are the same in different rats.

Complex structure of some relapse strains. Ehrlich (1909) pointed out that, while most trypanosome strains, which he called 'Unios,' are susceptible to the action of a single antibody, there are others, so-called 'Binios,' which are apparently not destroyed by, say, A or B antibody separately, but are destroyed when acted upon by A and B together. He explained this by supposing

that the 'Binio' strains comprise individuals whose protoplasm contains two distinct groups of receptors, as against a uniform group possessed by the more common 'Unio' strains. A simpler explanation for the phenomenon is that of Ritz (1914, 1916), who declared that a relapse strain may be composed of a mixture of two or more immunologically distinct types of trypanosome. This would also account for the fact that, while the immune serum against one particular strain may be equally effective against another, the reverse does not necessarily hold. Thus, a mouse immunized against the mixed strain AB will also be refractory to infection by strain A, but conversely a mouse immunized against strain A will not be protected against AB, since it will contain no antibody to hinder trypanosomes B from multiplying. Schilling and Neumann (1932) and Schilling, Schreck, Neumann and Kunert (1936) have given several examples of relationships of this kind between two strains, but these workers are not content with the explanation that one of the strains concerned may be a mixture of two separate types, and they interpret such cases as evidence that the antigenic property of a trypanosome cell and its receptor (sensitivity to antibody) are characters which have no dependence on one another.

PRESENT INVESTIGATIONS

In a previous communication (Lourie and O'Connor, 1936), we have shown that trypanolysis *in vitro* by mouse immune serum is very readily demonstrable, particularly in the presence of added complement. We decided to make use of this phenomenon as a means towards the detailed study and comparison of a strain of *T. rhodesiense* with organisms of the same strain derived from a number of first relapses in treated but uncured mouse infections.

The strain used was isolated from a case of sleeping sickness in 1923, and has since been maintained continuously in mice by blood inoculation. From this parent stem we obtained a series of 21 first relapse strains, by treating a number of mouse infections with a subcurative dose of 0.02 or 0.04 mgm. halarsol per 20 gm. mouse. In the great majority of the infections so treated, trypanosomes reappeared 5 to 24 days later; from each such case a relapse strain was secured and was maintained in mice. These are referred to as strains 1-21. In addition, we have studied a strain which we call a 'spontaneous variant,' which arose as follows: 32 mice were inoculated with equal amounts of mouse blood, containing a moderately large number of parasites of the parent stem, in citrated saline solution. With one exception, the anticipated course of infection resulted, trypanosomes appearing in the blood after an incubation period of 2 or 3 days. In the exceptional case, parasites appeared only after 7 days. An incubation period of more than 6 days in mice is extremely uncommon with a pathogenic trypanosome strain of this type, maintained continuously in mice; in fact, with the particular strain of *T. rhodesiense* with which we are working, such a prolonged incubation period has occurred only

5 times in 5,000 blood inoculations, during the past 8 years.* Even in mice into which one single trypanosome was injected (by the technique to be mentioned below), of 10 such cases in which infection resulted the incubation period was only 4 days in 4 instances, and 5 days in the remaining 6 cases. From the exceptional infection with the prolonged incubation period, which appeared in the group of 32 mice referred to above, the parasites were maintained as a separate strain. This was studied in detail together with the 21 relapse strains, and, as will be shown below, was found to differ from the parent stem in exactly the same way as is characteristic of relapse variants. One other of the 5 exceptional cases with prolonged incubation period, among the last 5,000 infections, was examined, and there also it was found that the parasites which did finally appear were of the relapse strain type. It would seem, then, that very rarely a mouse is able spontaneously to offer an appreciable resistance against the inoculated parasites, and that, as in the case of the immunity induced by drug treatment of an infection, the strain overcomes this resistance by emerging as an immunological variant.

The 22 variants studied were obtained on different dates between February, 1936, and January, 1937. In February, 1937, i.e., at periods ranging between 1 month and 1 year since isolation, an experiment was performed with the object of examining their relationship to one another and to the parent strain. A stock of immune serum was prepared against each strain, including the parent stem, and each serum was then examined for its trypanolytic activity *in vitro* against the homologous and against every other strain in turn. The method of obtaining the immune sera was as follows: Ten mice were infected with each strain; 2 days later, when there were 1 to 40 trypanosomes per field, the infections were treated by 0.08 mgm. halarsol per 20 gm. mouse. After a further 5 days, by which time there would be a relatively high lysin content in the blood (see Lourie and O'Connor, 1936), the mice were killed, and their sera pooled. The trypanolytic power of the various immune sera was tested against each of the strains by preparing a washed suspension of trypanosomes in guinea-pig medium (equal parts of fresh unheated guinea-pig serum and Ringer-glucose solution), and sowing 1/40th c.cm. of this suspension into each of a series of tubes containing 0.45 c.cm. of varying dilutions of the particular immune serum in guinea-pig medium. The suspension used for sowing was such that the concentration of trypanosomes in each tube at the outset should be about 1,000 per c.mm. The tubes were then incubated for half an hour at 37° C., after which their contents were examined microscopically for evidence of

* These 5,000 inoculations do not, of course, represent only routine passages for maintaining the strain during the period of 8 years; they include a very large proportion of inoculations in connection with numerous experiments. If one considers a line of the strain transmitted from one mouse to another twice a week, then 1,000 infections would occupy about 10 years. Therefore, in passage through a succession of single mice, an incubation period of 7 days or more would occur only once in about 10 years.

trypanolysis. In the results of this experiment the 23 sera invariably proved to be trypanolytic against the homologous organisms; the cross-tests with heterologous strains gave results falling into 3 categories, typical examples of which are given below (Table I—a, b and c).

TABLE I

Showing 3 types of effect of an immune serum upon heterologous trypanosomes *in vitro* :—

(a) Lysis of strain 8 by immune serum 5, in dilutions up to 128 in medium containing fresh guinea-pig serum.

(b) Lysis only of *some* trypanosomes of strain 13 by immune serum 5, in dilutions up to 128 in above medium.

(c) No lysis of strain 6 in any dilution of immune serum 5, in above medium.

	Trypanosomes tested	Dilution of immune serum 5	No. of living trypanosomes per 256 squares of the haemocytometer scale	
			Start	After half an hour at 37° C.
a	Strain 8	1 : 2	54	0
		1 : 8		0
		1 : 32		0
		1 : 128		0
		1 : 512		40
		1 : 2,048		58
b	Strain 13	1 : 2	50	7
		1 : 8		4
		1 : 32		6
		1 : 128		5
		1 : 512		54
		1 : 2,048		60
c	Strain 6	1 : 2	60	60
		1 : 8		72
		1 : 32		58
		1 : 128		66

TABLE II

Showing results of cross-tests for trypanolysis *in vitro* between trypanosomes and immune sera of 21 relapse strains, 1 'spontaneous variant,' and the parent stem of *T. rhodesiense*

Strain	Trypanosomes tested																					P	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21		's.v.'
Type*	a	p	a	abc	b	d	e	b	b	f	bc	g	bh	ci	b	h	bd	a	hj	bh	k	hl	p
1	a	32	0	32	32	0	0	0	0	0	0	0	0	0	0	0	0	32	0	0	0	0	0
2	p	0	512	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	512
3	a	128	0	128	128	0	0	0	0	0	0	0	0	0	0	0	0	128	0	0	0	0	0
4	abc	32	0	32	32	8	0	8	8	0	8	0	8/0	8/0	8	0	8	32	0	8	0	0	0
5	b	0	0	0	128	0	0	128	128	0	128/0	0	128/0	0	128	0	128	0	128	0	0	0	0
6	d	0	0	0	0	128	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
7	e	0	0	0	0	0	128	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
8	b	0	0	0	128	0	0	128	128	0	128/0	0	128/0	0	128	0	128	0	128	0	0	0	
9	b	0	0	0	128	0	0	128	128	0	128/0	0	128/0	0	128	0	128	0	128	0	0	0	
10	f	0	0	0	0	0	0	0	512	0	0	0	0	0	0	0	0	0	0	0	0	0	
11	bc	0	0	0	128	0	0	128	128	0	128	0	128/0	32/0	128	0	128	0	128	0	0	0	
12	g	0	0	0	0	0	0	0	0	0	0	128	0	0	0	0	0	0	0	0	0	0	
13	bh	0	0	0	128	0	0	128	128	0	128/0	0	128	0	128	128	128	0	128/0	128	0	+/0	
14	ci	0	0	0	0	0	0	0	0	0	128/0	0	0	128	0	0	0	0	0	0	0	0	
15	b	0	0	0	128	0	0	128	128	0	128/0	0	128/0	0	128	0	128	0	128	0	0	0	
16	h	0	0	0	0	0	0	0	0	0	0	0	+/0	0	0	128	0	0	128/0	0	0	+/0	
17	bd	0	0	0	128	128	0	128	128	0	128/0	0	128/0	0	128	0	128	0	128	0	0	0	
18	a	32	0	32	0	0	0	0	0	0	0	0	0	0	0	0	0	32	0	0	0	0	
19	hj	0	0	0	0	0	0	0	0	0	0	0	+/0	0	0	512	0	0	512	0	0	+/0	
20	bh	0	0	0	128	0	0	128	128	0	128/0	0	128	0	128	512	128	0	512/0	128	0	+/0	
21	k	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	128	0	
's.v.'	hl	0	0	0	0	0	0	0	0	0	0	0	+/0	0	0	128	0	0	128/0	0	0	128	
P	p	0	512	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	512

1-21 = Relapse strains numbered consecutively, in chronological order of their isolation between February, 1936, and January, 1937.
's.v.' = 'spontaneous variant,' P = parent stem.

* Type of organisms in the various strains, as determined on the basis of experiments and conclusions described in the text. Types a-l; p = parent stem type. Two or three letters together indicate mixtures of two or three types in one strain; underlining of one letter indicates that trypanosomes of that type are present in very considerable excess, in the particular strain.

The following are examples of the manner in which results are to be read:—
128 = as far as microscopical examination would indicate, there was lysis of all the trypanosomes, by immune serum in dilutions up to 128 (in presence of complement).

128/0 = lysis only of *some* trypanosomes by immune serum in dilutions up to 128 (in presence of complement).

+ /0 = lysis only of *very few* trypanosomes by immune serum diluted in complement. Dead individuals seen, but relative number destroyed was so small that it was difficult to determine the actual titre of the immune serum against the susceptible type in the mixture.

0 = no apparent lysis of trypanosomes in any dilution of immune serum, in the presence of complement.

(a) There might be lysis apparently of *all* organisms, in concentrations of immune serum down to a certain level, the latter depending on the titre of lysin in the particular serum.*

(b) Within the effective range of the immune serum only *some* of the organisms might be destroyed.

(c) The third type of result is where no lysis occurs in any concentration of the immune serum.

The results shown in sections a, b and c of Table I may conveniently be expressed as follows :—

For section a—the single number 128 (this being the lysin titre of immune serum 5 against the trypanosomes of strain 8).

For section b—128/0 (indicating that, in the case of trypanosomes 13, immune serum 5 is effective, to a titre of 128, against only *some* of the parasites, others being unaffected in any dilution).

For section c — 0 (apparently no organisms of strain 6 being lysed by immune serum 5).

Expressing in similar manner all the results of the 529 cross-tests between the trypanosomes of the 23 strains (including the parent line) and the corresponding immune sera, these findings have been gathered together in a single Table (Table II).

Analysing this Table, we find that it consists of what may be regarded as a combination of a number of examples of 3 simpler type Tables. In order to explain Table II, therefore, we will extract from it the 3 type Tables (Tables IIA, IIB and IIC), each of which will be discussed and interpreted separately below.

Table IIA. The explanation of the relationships shown in this Table offer no difficulty. There is reciprocity between any two strains, in that, if the immune serum for one be lytic against the trypanosomes of the other, then the immune serum for the latter likewise destroys the trypanosomes of the former strain. Thus, in the example of Table IIA, on the one hand the immune sera, and on the other hand the trypanosomes, of strains 1 and 3 are interchangeable, and these strains may therefore provisionally be considered as identical, until further tests bring out any possible differences. (That it would be wrong, however, on the basis of this restricted Table, finally to assume that these two strains are exactly alike, will be explained in the discussion on Table IIC). The immune sera of 1 and 3 do not lyse the trypanosomes of strain 5, and,

* Sometimes we encountered the form of 'zone' effect to which attention has already been drawn (Lourie and O'Connor, 1936), and which we gave reasons for believing to be due to an anti-complementary property of mouse serum. In these cases lysis was somewhat delayed in higher concentrations of immune serum, while being complete within half an hour in lower concentrations. However, when this phenomenon was observed, even in half an hour in the higher concentrations of immune serum, the trypanosomes were reduced in numbers, damaged and sluggish, and within a few hours were all destroyed.

TABLE IIA

Showing results of cross-tests for lysis between 3 relapse strains and their corresponding immune sera

Interpretation : Strains 1 and 3 are similar to one another (type *a*), while strain 5 is different (type *b*).

		Trypanosomes		
		1	5	3
Immune sera	Type*	<i>a</i>	<i>b</i>	<i>a</i>
	1	L	O	L
	5	O	L	O
	3	L	O	L

* Type of trypanosomes in the strains tested, as determined by reasoning and experiments detailed in the text.

L = lysis apparently of all organisms, within effective range of the immune serum.

O = no appreciable lysis.

conversely, immune serum 5 has no effect on trypanosomes 1 or 3. Strain 5 is, therefore, clearly of a different type from that of the other two. Assuming, for convenience, then, until differences are revealed between strains 1 and 3, that these are identical with one another, we may consider them as representing a common trypanosome type *a*. The organisms of 5 being proved already to be different, we refer to them as representatives of type *b*.

Table IIB. An examination of this Table immediately suggests that strain 11 includes a mixture of trypanosomes of two distinct types, one of which is similar to that of strain 5. The immune serum for the latter destroys only *some* of the organisms of strain 11, whilst immune serum 11 lyses apparently all the trypanosomes of strain 5. Since strain 5, then, has been referred to as being of type *b*, we will refer to strain 11 as type *bc*, thus indicating a mixture of organisms of types *b* and *c*.

TABLE IIB

Showing results of cross-tests for lysis between 2 relapse strains and their corresponding immune sera

Interpretation : Strain 11 is a mixture of some individuals similar to those of strain 5 (type *b*) with others of a different type (type *c*).

		Trypanosomes	
		5	11
		<i>b</i>	<i>bc</i>
Immune sera	Type*		
5	<i>b</i>	L	L/O
11	<i>bc</i>	L	L

* Type of trypanosomes in the strains tested, as determined by reasoning and experiments detailed in the text.

L = lysis apparently of all organisms, within effective range of the immune serum.

L/O = lysis only of *some* organisms, within effective range of the immune serum.

The theoretical interpretation of Table IIB as showing that strain 11 is composed of trypanosomes of at least two separate types, is substantiated by the following experiment :—

Experiment : Two mice were infected by inoculating each with a single trypanosome of relapse strain 11, by a method similar to that of Oehler (1913). This method consists in drawing up into a capillary of minute bore a small amount of dilute mouse serum containing trypanosomes, whose concentration has previously been suitably adjusted. The capillary is then placed on a slide and examined with the 1/6th objective until a single trypanosome is found, and a small length, of about 0.5 to 1 cm., containing this trypanosome is broken off. The small piece is then inserted in the lumen of a hypodermic needle, which is fitted to a syringe containing about 0.3 c.cm. saline, and the capillary thus injected intraperitoneally into a mouse.

Having in this way obtained from strain 11 two separate single-cell infections, the trypanosomes of which we may now call 11(*i*) and 11(*ii*), the next step was to examine the lytic power of immune serum 5 against trypanosomes 11, 11(*i*) and 11(*ii*) respectively.

The result of this experiment is given in Table III, which shows that the two single-cell lines derived from strain 11 are of two quite distinct types, since immune serum 5, active against only some of the organisms of strain 11, is entirely without effect on 11 (*i*), while being lytic to a titre of 128 against 11 (*ii*). It should be pointed out in this place that no type differences between one another could be detected among 10 single-cell strains isolated, by the above technique, from the parent stem.

TABLE III

Showing *in vitro* effect of strain 5 immune serum upon the trypanosomes of strain 11, and upon two single-cell lines derived from the latter

Trypanosomes tested	Dilution of immune serum 5 in medium containing complement	No. of living trypanosomes per 256 squares of the haemocytometer scale	
		Start	After half an hour at 37° C.
Strain 11	1 : 2	92	46
	1 : 8		40
	1 : 32		35
	1 : 128		43
	1 : 512		90
	1 : 2,048		96
Single-cell line 11 (i)	1 : 2	84	76
	1 : 8		90
	1 : 32		82
	1 : 128		74
	1 : 512		80
	1 : 2,048		72
Single-cell line 11 (ii)	1 : 2	56	0
	1 : 8		0
	1 : 32		0
	1 : 128		0
	1 : 512		40
	1 : 2,048		62

It is easily possible with two such strains as 5 and 11 to reproduce one of the types of experiment upon which Schilling and Neumann (1932) depend for their conclusion that the antigenic property of a trypanosome cell and its receptor are characters which have no dependence on one another, and such an experiment

is described immediately below. However, the *in vitro* experiment of Table III above shows that it is unnecessary in this case to look for an explanation other than that offered by Ritz (1914), namely, that one of the strains concerned is composed of trypanosomes of at least two distinct types.

Experiment : Several mice infected with strains 11 and 5 respectively. Cured by halarisol. After one week, reinfection by these strains attempted. Results :—

- (a) Mouse 11 reinoculated with strain 11 —> no reinfection.
 (b) " 11 " " 5 —> "
 (c) " 5 " " 5 —> "
 (d) " 5 " " 11 —> reinfected, with normal incubation period.

TABLE IIc

Showing results of cross-tests for lysis between 3 relapse strains and their corresponding immune sera

Interpretation : Strain 17 is a mixture of individuals of type *b*, together with a considerably smaller number of type *d*.

		Trypanosomes		
		17	5	6
Type*		<i>bd</i>	<i>b</i>	<i>d</i>
Immune sera	17	L	L	L
	5	L	L	O
	6	O	O	L

* Type of trypanosomes in the strains tested, as determined by reasoning and experiments detailed in the text.

L = lysis apparently of all organisms, within effective range of the immune serum.

O = no appreciable lysis.

Table IIc. In a strain which consists of a mixture of two types, these components may be found to occur in any ratio. For example, in strain 11

it is evident that they are present approximately in equal numbers (see upper section of Table III); in strain 13 the proportion must be about 10 to 1 (see section b, Table I). Suppose now a strain to be composed of trypanosomes $b:d::500:1$. If an immune serum be prepared against this compound strain in our usual way, by inoculating it into a mouse which is treated by drug two days later, we shall expect such a serum to be lytic against a strain of type b trypanosomes, since these formed an overwhelming proportion of the organisms stimulating the production of immune body in the infected mouse. The immune serum may equally be expected to be active against a strain of type d trypanosomes, in spite of the very small number of those organisms which were present in the mouse forming the antibody. This statement is made on the authority of experiments such as one to be described later (pp. 333-334), from which it will be seen that when a mouse is treated for an infection with a mixture of trypanosomes, in which individuals of one type are even 1,600 times less numerous than those of another, the immune serum produced may be lytic against organisms of the one type as well as against those of the other. An immune serum against our compound strain will, then, be expected to be active against the homologous combined strain, and also against strains composed solely of organisms of type b and type d respectively. Now, an immune serum prepared against trypanosomes b alone may likewise *appear* to be completely lytic *in vitro* towards the compound strain, since the relatively few type d survivors will easily be missed on microscopical examination. However, if immune serum d be brought to act on the compound strain, there will apparently be no lysis, for the relative number of trypanosomes destroyed would be too small to be noticed. The relationship between the compound strain and its two components would, in fact, be that expressed in Table IIc. This Table is explained, then, on the basis that strain 17 consists of a relatively large number of trypanosomes of the same type as strain 5 (type b), together with a relatively much smaller number of the type of strain 6 (type d). We will therefore describe strain 17 as being of type bd , and by underlining the letter b we indicate that organisms of this type are present in much greater numbers than those of type d .

In order to prove that the above interpretation of Table IIc is correct, the following experiment was performed, in which the two components of strain 17 were separated and clearly identified with strain 5 (type b) and with strain 6 (type d) respectively.

Experiment: Washed trypanosomes of strain 17 were suspended, in concentration of about 1,000 per c.mm., in 2 tubes, one of which contained 0.5 c.cm. of immune serum 5 (type b) and the other the same amount of immune serum 6 (type d), each diluted with an equal volume of fresh guinea-pig serum. After incubation at 37° C. for half an hour, the survivors in the two tubes were washed once in Ringer-glucose solution, and injected respectively into mouse 1 and mouse 2. When the resulting infections were at their height, the trypanosomes were removed from these mice and examined respectively for their susceptibility to immune sera 17, 5 and 6, with the results shown in Table IV.

TABLE IV

Showing effect of immune sera 17 (type *bd*), 5 (type *b*), and 6 (type *d*) upon the trypanosomes of mice 1 and 2. Mouse 1 had been inoculated with the survivors after *in vitro* contact of strain 17 with immune serum 5, and mouse 2 with survivors after contact of the same strain with immune serum 6

Serum tested	Trypanosomes tested from	
	Mouse 1	Mouse 2
17 (type <i>bd</i>)	lysis	lysis
5 (type <i>b</i>)	no lysis	lysis
6 (type <i>d</i>)	lysis	no lysis

It may be seen from Table IV that, following the preliminary *in vitro* contact of trypanosomes 17 with immune serum 5 (type *b*), the surviving organisms (inoculated into mouse 1) were subsequently proved to be similar to strain 6 (type *d*). On the other hand, after *in vitro* contact of the trypanosomes 17 with immune serum 6 (type *d*), the survivors in this case (inoculated into mouse 2) proved to be similar to strain 5 (type *b*).

A study of Table IIc will point to the reason for the statement made in discussing Table IIA, that it would be wrong to assume complete identity between any two strains on the basis of their respective immune sera apparently being completely lytic equally against the parasites of either strain. Thus, if examination had been confined to the trypanosomes and immune sera of strains 17 and 5, one might have been tempted to conclude that these strains were identical, but differences between them are revealed on including a third strain, no. 6, into the cross-tests.

Before passing on, special attention should be drawn to Table V below, which conforms to type Table IIc, and which, like the latter, has also been extracted from Table II. There is, however, a special feature in this case. It may be seen from Table V that, on the analogy of Table IIc, strain 4 evidently is a mixture of a large number of trypanosomes of the type of strain 1 together with a much smaller number of strain 11 organisms. But strain 11 has already been shown (Tables IIB and III) to include, in about equal numbers, organisms of 2 dissimilar types. Therefore, strain 4 must be composed of at least 3 distinct trypanosome types.

On the basis of the interpretations placed on Tables IIA, IIB and IIc, it has been possible, then, to determine the probable type-category of each of the 23 strains tested against one another, the results of which are gathered together on Table II. Next to the identification number of each strain, on this Table we

TABLE V

Showing results of cross-tests for lysis between strains 4, 1 (type *a*), and 11 (type *bc*), and their corresponding immune sera

Interpretation: Strain 4 is a mixture of trypanosomes of type *a*, together with a considerably smaller number of type *bc*. It therefore contains at least 3 distinct types.

		Trypanosomes			
		4	1	11	
Immune sera	Type*	<u>abc</u>	<i>a</i>	<i>bc</i>	
	4	<u>abc</u>	L	L	L
	1	<i>a</i>	L	L	O
	11	<i>bc</i>	O	O	L

* Type of trypanosomes in the strains tested, as determined by reasoning and experiments detailed in the text.

L = lysis apparently of all organisms, within effective range of the immune serum.

O = no appreciable lysis.

have indicated, by the appropriate letters, the presumptive type to which it conforms, and it may be seen that in every single case the result is adequately explained by the typing adopted for the particular strains concerned. The following are certain points which emerge from Table II :—

1. In 22 relapse strains (including the 'spontaneous variant,' which, as mentioned before, behaves like a relapse strain) 13 distinct immunological types appeared.

2. In 7 of the strains there was a mixture of 2, and in one strain a mixture of 3, separate types. The effect of this is that altogether there were 16 different types or combinations of types in the 22 relapse strains.

3. Certain types tend to appear in relapse strains more frequently than others, as the following analysis shows :—

Type *b* appeared in 9 of the 22 strains.

" <i>h</i>	"	5	"	22	"
" <i>a</i>	"	4	"	22	"
" <i>c</i>	"	3	"	22	"
" <i>d</i>	"	2	"	22	"

Types *e, f, g, i, j, k, l* and *p* each appeared only in 1 " 22 "

Thus, of the 13 types, one (type *b*) appeared in as many as 9 of the 22 relapse strains, whereas 8 other types occurred only once each.

The factors determining whether one or more types are to appear in the trypanosomes of a relapse, and which particular types these are to be, are quite obscure. Such variables as the date of treatment, size of the subcurative drug dose, degree of infection at the time of treatment, and the duration of the latent period between treatment and relapse, have been excluded. Thus, strains 4 to 7 were of types *abc*, *b*, *d* and *e* respectively, although they arose in relapses of 4 mice which were all treated at the same time, by the same dose of drug, when there were about 30 trypanosomes per field in each infection, the relapses all following 9 to 12 days after treatment. Conversely, strains 1 and 18 were both of the same type, *a*, though there was an interval of 11 months between their isolation; the former strain was derived from a mouse treated when trypanosomes were swarming, the relapse occurring 10 days later, and the latter strain came from a mouse treated when there were only a few trypanosomes per field, the relapse taking place after 17 days. Another strain, no. 3, also of type *a*, sprang from an infection which relapsed only 5 days after treatment.

4. One of the relapse strains (no. 2) proved to be of the same type (*p*) as the parent stem. The significance of this will be discussed later.

5. With one notable exception (serum 4) the titres of the immune sera all fell between 32 and 512, being 128 in most cases. This range is in agreement with our earlier records (Lourie and O'Connor, 1936) of immune serum titre in mice 5 days after treatment of infections with various pathogenic trypanosomes at our disposal. It will be noted that the exception in the present series occurred in the case of the immune serum for the only strain (no. 4) which may definitely be presumed to comprise at least 3 trypanosome types. The serum, while being effective against type *a* organisms in a dilution of 32, was lytic against types *b* and *c* only to a titre of 8. The question arises, therefore, (*i*) whether this very low titre is to be accounted for by the antibody mechanism of the host being unduly embarrassed on being confronted simultaneously with 3 antigenically different trypanosomes,* or (*ii*) whether the organisms of types *b* and *c* were present in such very small numbers as to afford only a slight stimulus to production of the corresponding antibodies. With the object of investigating this point, the following experiment was performed:—

Experiment: Four compound strains were artificially produced by mixing *in vitro*, in differing proportions, trypanosomes of 3 distinct types, viz. :—type *a* (from strain

* In his 'Outline of Immunity,' Topley (1933) quotes Benjamin and Witzinger (1912), and Huntoon and Craig (1921), whose work suggests that the injection of more than one antigen, simultaneously or in rapid succession, lessens the production of one or other of the corresponding antibodies. Topley also quotes, for the particular case of antitoxins, the careful work of Glenny and his colleagues (1925*a*, 1925*b*, 1926, 1928), which brings out the same point, and from which it appears to be likely that this 'crowding out' effect is particularly prone to occur when any one antigen is present in great excess.

1), type *b* (from strain 5), and type *d* (from strain 6). The approximate ratio of organisms of each type in the 4 mixed strains was as follows:—

Mixture 1 — type <i>a</i> : type <i>b</i> : type <i>d</i> ::	1 : 1 : 1.
2 — " " "	:: 100 : 1 : 1.
3 — " " "	:: 400 : 1 : 1.
4 — " " "	:: 1,600 : 1 : 1.

The next step was to prepare an immune serum against each of these artificially mixed strains, by the same method as was used for the main experiment, i.e., by inoculating 3 or 4 mice with each mixture, treating with an adequate dose of halarol 2 days later, and bleeding and pooling the serum for each particular group after a further 5 days. The immune serum corresponding to each mixed strain was then tested for its trypanolytic power against the homologous trypanosomes (which had been kept by passage through mice), and against separate strains of each of the three component types. The results are given in Table VI.

TABLE VI

Showing results of an experiment in which trypanosomes of 3 distinct types were mixed in varying proportions, and immune sera prepared against each of 4 compound strains thus produced; the respective immune sera were then tested against the homologous mixed strain, and against each of the component types

Immune serum tested	Trypanosomes tested	Titre of immune serum
Mixture 1 (types <i>a</i> : <i>b</i> : <i>d</i> :: 1 : 1 : 1)	Mixture 1	32
	type <i>a</i>	32
	" <i>b</i>	128
	" <i>d</i>	128
Mixture 2 (types <i>a</i> : <i>b</i> : <i>d</i> :: 100 : 1 : 1)	Mixture 2	128
	type <i>a</i>	128
	" <i>b</i>	32
	" <i>d</i>	128
Mixture 3 (types <i>a</i> : <i>b</i> : <i>d</i> :: 400 : 1 : 1)	Mixture 3	32
	type <i>a</i>	32
	" <i>b</i>	8
	" <i>d</i>	32
Mixture 4 (types <i>a</i> : <i>b</i> : <i>d</i> :: 1,600 : 1 : 1)	Mixture 4	128
	type <i>a</i>	128
	" <i>b</i>	8
	" <i>d</i>	32

It may be seen from Table VI that the mere mixture, in one strain, of three antigenically different types does not of itself result in the homologous immune serum having an abnormally low titre against each separate component type (see results for immune sera of mixtures 1 and 2). When, however, one of these components is present in the proportion of 1 to 400, or less (mixtures 3 and 4), then the immune serum of the compound strain may be lytic against that type to a titre of only 8.

The conclusion that types *b* and *c* were in fact present in strain 4 in extremely small numbers is further supported by the fact that the organisms of these types disappeared from the strain in the course of subinoculation during the next few months. Thus Table VII shows the results of several series of tests for lysis

TABLE VII

Showing results of tests for trypanolysis *in vitro*, against various strains, by immune serum for strain 4 obtained and examined on 3 different dates

Date when immune serum 4 was obtained and tested	Apparent type components of strain 4	Trypanosomes tested (strain no. and type)											
		1	2	3	4	5	6	7	8	9	10	11	P
		<i>a</i>	<i>p</i>	<i>a</i>	*	<i>b</i>	<i>d</i>	<i>e</i>	<i>b</i>	<i>b</i>	<i>f</i>	<i>bc</i>	<i>p</i>
11.1.37	<i>abc</i>	32	0	32	32	8	0	0	8	8	0	8	0
17.2.37	<i>abc</i>	32	0	32	32	8	0	0	8	8	0	8	0
20.4.37	<i>a</i>	32	0	32	32	0	0	0	0	0	0	0	0

* Type *abc* on 11.1.37 and 17.2.37; type *a* on 20.4.37
Results to be read in same way as in Table II

between immune serum 4 and various strains, the immune serum having been obtained and examined on three different dates, viz.: January 11th, February 17th and April 20th, 1937. It may be seen that, between February and April, types *b* and *c* must have been lost by strain 4, since its immune serum on the last date was no longer lytic towards those strains which were composed of type *b*, or types *b* and *c*.

As stated in the Introduction, Rosenthal (1913) and Ritz (1914) have already pointed out that one trypanosome type in a strain may, in the course of passage through mice, overgrow and finally displace another, this being the explanation offered for the fact that a relapse strain may, in time, revert to the parent strain type. In these cases the relapse strain is held to arise in the first place as a mixture of organisms of the parent and the relapse type, the latter eventually being displaced. This explanation is supported by our experience with relapse

strain 2. When immune serum for this strain was first obtained and tested, about 2 weeks after isolation, in June, 1936, it was found to be lytic against certain other of the relapse strains, which had been isolated at about the same time, and *also* against the parent stem. This was again found to be the case when immune serum for the strain was prepared and tested after about another 2 weeks. Up to this time, therefore, relapse strain 2 evidently contained a mixture of organisms of the parent and the relapse type. However, when immune serum was prepared and tested after a further 4 months, it was then found to lyse only the parent, and to be quite without effect against any of the relapse organisms (other than those of the homologous strain). This was confirmed in repeated subsequent tests, of which the result included in Table II is one example. It is evident, therefore, that, within about 5 months of its isolation, the relapse type components had disappeared from strain 2, leaving it to be indistinguishable from the parent stem.

Apart from such changes in a strain as are accountable to variation in the relative numbers of two or more different types, the present work confirms that a strain retains its immunological type, if not indefinitely, then at least for very long periods, on passage through mice. Thus, the earliest relapse strain to be isolated, strain 1, was obtained in February, 1936; when examined in cross-tests with the parent stem 15 months later, it was found still to be distinct from the latter. However, it should not immediately be assumed that this is proof of the two strains having both remained true to type during the period concerned. What evidence have we that each strain might not have altered from time to time, so that after 15 months we do no more than register differences between two trypanosome types which are themselves different from the two types compared at the outset? There are three considerations which render this extremely unlikely:—

1. As has already been pointed out (pp. 321-322) in the two instances in which 'spontaneous variants' have been detected, it is noteworthy that, in the actual infections in which the variations occurred, the incubation periods were in both cases exceptionally prolonged, namely, 7 days. During the examination of many hundreds of immune sera in the past year and a half we have not once, in spite of the ample opportunity, encountered a 'spontaneous variant' arising in a parent or relapse strain infection of normal incubation period. It seems justifiable, therefore, to associate the emergence of a 'spontaneous variant' with an incubation period of about 7 days or more; and, since such incubation periods have occurred only once in about 1,000 passages, it is a reasonable conclusion that 'spontaneous variation' is a correspondingly exceptional occurrence. It should be mentioned that a 'spontaneous variant' evidently remains different in type from the parent strain for protracted periods, no less than does a true relapse strain. The 'spontaneous variant' referred to in most of the work of this paper was, like relapse strain 1, obtained in February, 1936, and 15 months later was still found to be distinct from the parent stem.

2. Three of the relapse strains, nos. 5, 8 and 9, were compared in November, 1936, and proved all to be of the same type, *b*. When retested 6 months later, they were found still to be indistinguishable from one another. If sporadic alteration in type were liable to occur with any frequency, then it is extremely improbable that the three strains would again prove to be identical at the later examination, for this would involve the coincidences that all three strains had altered in type during the period concerned, and that the three new types adopted had all happened again to be identical with one another. Relapse strains 1 and 3 (type *a*), likewise, were compared with one another on two occasions, with an interval of 6 months, and these also were found again to be indistinguishable on the second examination.

3. If an immune serum against a particular strain were to be preserved for a long period, and were then tested and found still to be lytic against that strain, and not against controls of a different type, this would be very strong evidence of the stability of trypanosome type in the strain. An experiment of this nature was performed, as follows :—

Experiment : Two samples of immune serum, one against the parent stem and one against strain 5 (type *b*), were preserved in the ice-box for periods of 7 and 5 months respectively. Each serum was then examined for its trypanolytic power against the homologous and against the other strain, with the following results :—

Parent serum	+	parent trypanosomes	—>	lysis (titre - 128).
"	"	+ strain 5	"	—> no lysis.
Strain 5	"	+ " 5	"	—> lysis (titre - 64).
" 5	"	+ parent	"	—> no lysis.

It may be seen that the trypanosomes of the parent stem and of strain 5 remained specifically susceptible to homologous immune sera which had been prepared respectively 7 and 5 months before. The strains, therefore, had presumably not altered in type during these periods.

Incidentally, the above experiment gives further information upon the stability of the trypanocidal antibody, on storing in the ice-box. In our previous report (Lourie and O'Connor, 1936), reference was made to the recent statement of Schilling, Schreck, Neumann and Kunert (1936) that immune serum must always be fresh for the demonstration of antibodies towards trypanosomes, and we reported observations which showed that mouse immune serum might be kept at 0° C. for periods up to 6 weeks without any loss in its potency. The two sera in the experiment described above were found to be no less active against the homologous trypanosomes after storage than they were when first prepared. Mouse immune serum may, therefore, be kept at 0° C. for at least 7 months without any impairment of its trypanocidal property.

SUMMARY AND CONCLUSIONS

A. It is well known that the trypanosomes of a relapse differ immunologically from those of the initial stages of an infection, and that the differences are most conveniently studied in strains which produce a rapidly fatal infection in mice. It has been held that, in these hosts, the steady multiplication of parasites, being opposed by no appreciable resistance, ensures that the immunological type of a strain remains unaltered for an indefinite number of passages. Working with a strain of *T. rhodesiense* which has been maintained continuously in mice for the past 14 years, we have found, however, that very rarely a mouse is actually capable of offering an appreciable resistance against infection, so that the incubation period is 7 days or more instead of being 5 days or less, as is normally the case even in infections produced by one single trypanosome. During the past 8 years, among 5,000 infections there have been only 5 with such prolonged incubation periods; in 2 of these the trypanosomes were studied and found to differ from the parent stem in the same way as is characteristic of relapse strains. It would appear, therefore, that 1 mouse in about 1,000 is able spontaneously to offer a resistance against the inoculated parasites, and that, as in the case of the immunity induced by drug treatment of an infection, the trypanosomes overcome this resistance by emerging with altered immunological characters. We refer to a strain that has arisen in this way as a 'spontaneous variant.'

B. Twenty-one first relapse strains were obtained by giving a subcurative dose of halarsol to mice infected with the 14-year-old mouse-passage *T. rhodesiense*. The relapse strains were separately maintained in mice, and, after periods ranging between one month and one year since their isolation, immune sera were prepared respectively against each strain and also against a 'spontaneous variant' and the parent stem. The method of obtaining immune serum was by infecting 10 mice with the particular strain, treating with halarsol 2 days later, and killing the mice and pooling their sera after a further 5 days. The respective immune sera were then tested for their trypanolytic action *in vitro* against the organisms of each strain in turn. Conclusions arising from these tests, and from further experiments suggested by the results obtained, are as follows:—

1. In the 22 relapse strains (among which we include the 'spontaneous variant') 13 distinct immunological trypanosome types appeared.

2. In 7 of the strains there was a mixture of two, and in 1 strain a mixture of three separate types, with the effect that altogether there were 16 different types or combinations of types in 22 strains. That a relapse strain may include more than one trypanosome type has been confirmed by isolating from a strain two single organisms, the progeny of one of which was then shown to be immunologically different from that of the other.

3. Certain types tend to appear in relapse strains more frequently than others. Thus, one appeared in as many as 9 of the 22 strains, whereas 8 other types occurred only once.

4. The factors determining whether one or more types are to appear in the trypanosomes of a relapse, and which particular types these are to be, are quite obscure. Such variables as the date of treatment, size of the subcurative dose, the degree of infection at the time of treatment, and the duration of the latent period between treatment and relapse have all been excluded.

5. One of the relapse strains proved to be of the same type as the parent stem. Experiments are described to support the view that such a strain arises in the first place as a mixture of relapse type individuals with others still of the parent type, the latter overgrowing and finally displacing the former, in the course of passage from mouse to mouse.

6. The immune serum produced by a mouse infected with a strain composed of several types is lytic against organisms of each of these types—even though, in the compound strain, the individuals of one type may have been only about $\frac{1}{1,600}$ times as numerous as those of another.

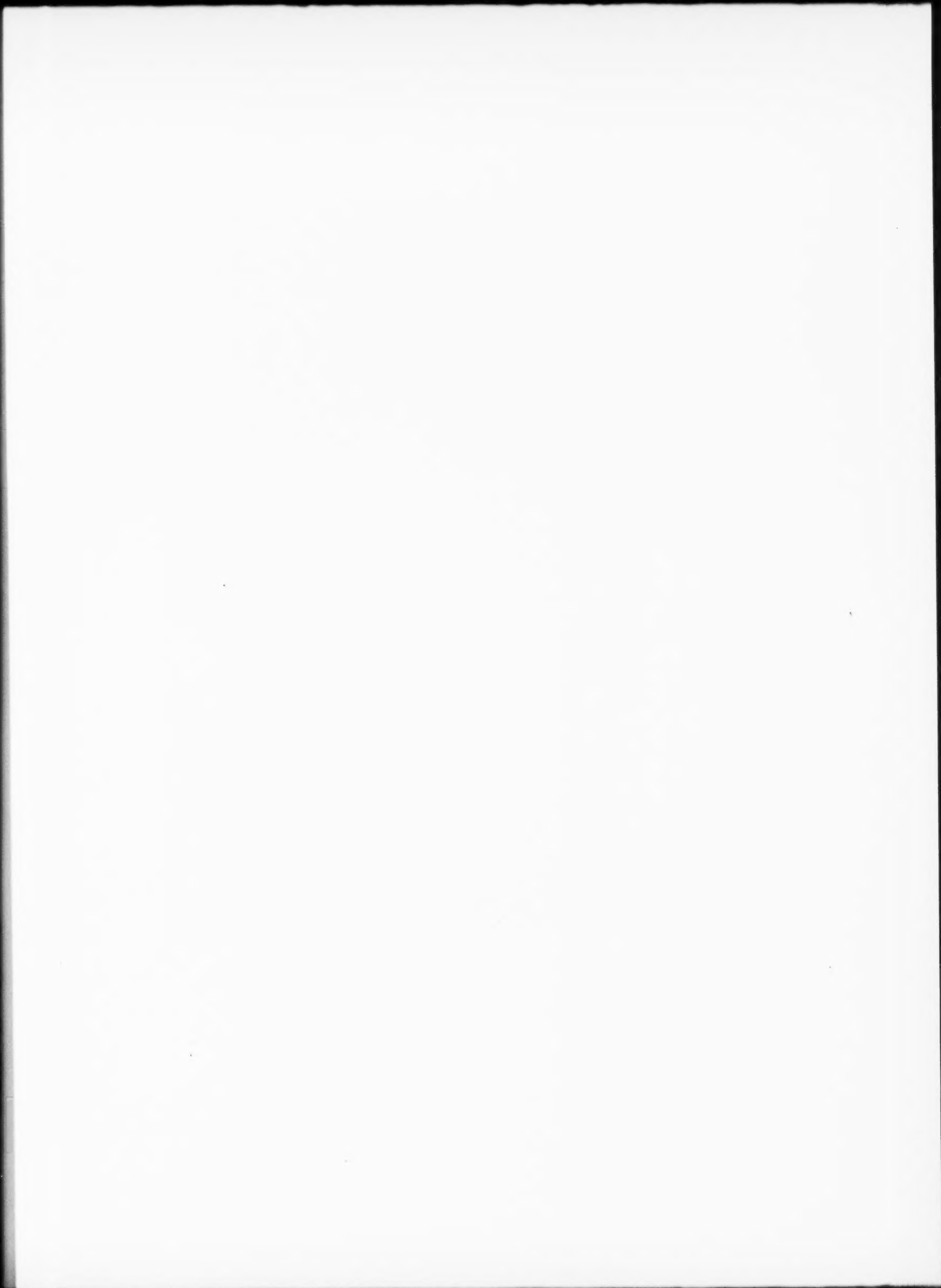
7. It is confirmed that, on passage through mice, the immunological type of the parent, or of relapse, trypanosomes remains fixed for prolonged periods; apparently the type may change but once in about 1,000 passages, i.e., once in about 10 years, in a strain inoculated from mouse to mouse at the rate of two passages per week. In strains comprising a mixture of trypanosome types, all the individuals of one or more of these types may at any time be lost, in the course of mouse passage.

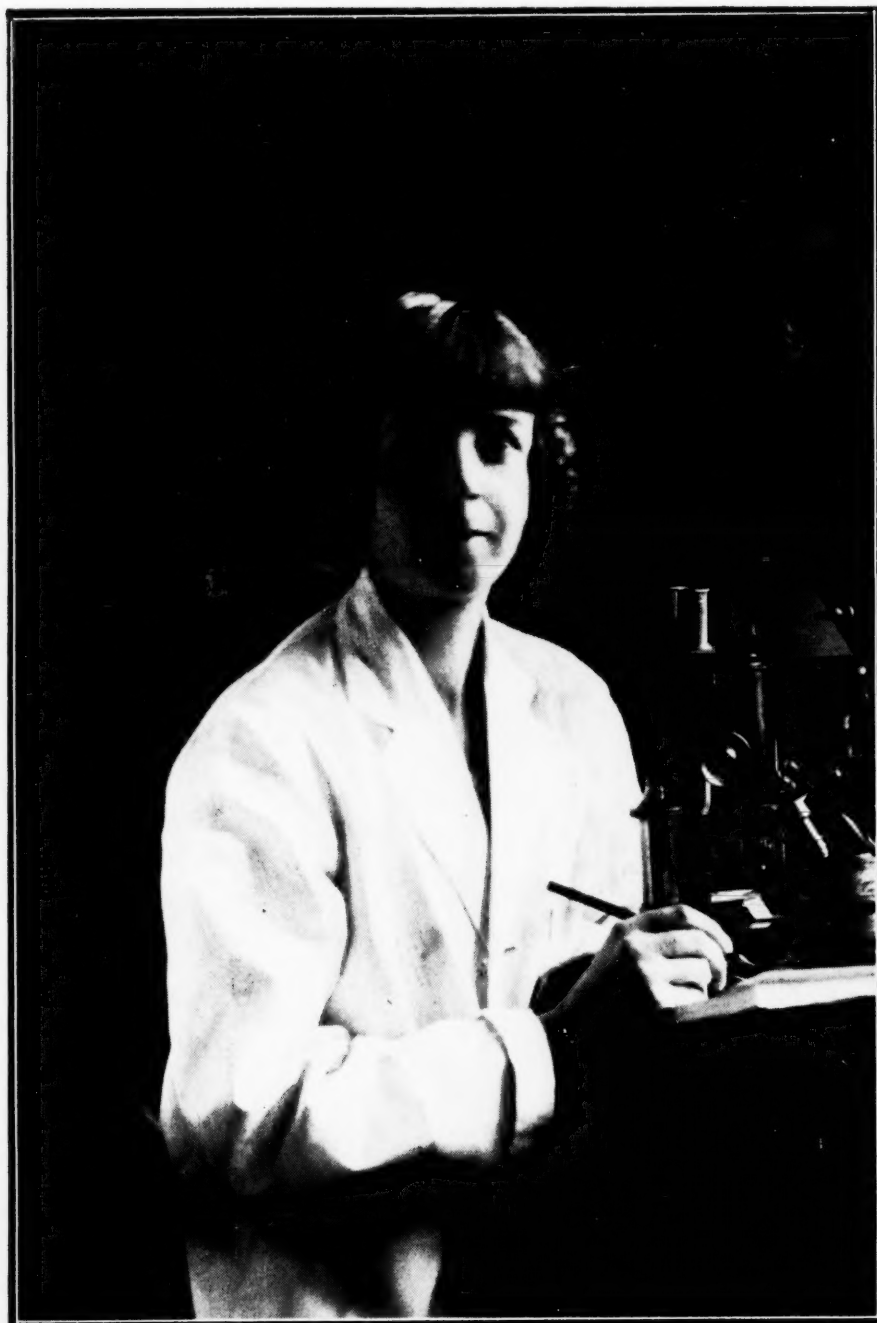
8. Mouse immune serum may be kept at 0° C. for at least 7 months without any impairment of its trypanocidal property.

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*By courtesy of
'The North Western Naturalist.'*

Alice M. Gowan.



IN MEMORIAM

The Editors regret to announce the death on August 8th, 1937, of MISS ALWEN MYFANWY EVANS, D.Sc., F.R.E.S.

Dr. Evans was appointed Lecturer in Entomology to the Liverpool School of Tropical Medicine in 1918, and from that time devoted herself especially to the study of mosquitoes. In 1925 she went to Sierra Leone to study mosquitoes and their breeding places, and in 1936 she was awarded a grant by the Leverhulme Research Committee, which enabled her to visit Kenya, where she conducted an investigation into certain problems connected with malaria-carrying mosquitoes.

She published many papers on entomology, as well as 'A Short Illustrated Guide to the Anophelines of Tropical and South Africa.' Shortly before her death she completed a monograph of the anopheline mosquitoes of the Ethiopian region.

STUDIES ON THE HIGHER DIPTERA OF MEDICAL AND VETERINARY IMPORTANCE

ILLUSTRATIONS OF THE TERMINALIA OF *COBBOLDIA ELEPHANTIS* STEEL, *C. CHRYSIDIFORMIS* RODHAIN AND BEQUAERT AND *RUTTENIA LOXODONTIS* RODHAIN

BY

W. S. PATTON

(From the Department of Entomology, Liverpool School of Tropical Medicine)

(Received for publication 24 June, 1937)

The genus *Cobboldia* contains three species whose larvae are parasites of the stomach of the elephant—those of *C. elephantis* in the stomach of the Indian elephant, and those of *C. chrysidiformis* and *C. loxodontis* in the stomach of the African elephant. The species, their early stages and life-histories have been fully described by Rodhain and Bequaert (1919), so that it is unnecessary for me to refer to them here. Through the generosity of Dr. Rodhain, who gave me one male and two females of *C. chrysidiformis*, I am able to illustrate the terminalia of this species. I have long had two females of *C. elephantis* bred from larvae collected from the Indian elephant in South India, and recently Mr. Pfaff, M.R.C.V.S., Veterinary Research Officer, Insein, Burma, bred out a male and very generously gave it to me, so that I am now able to describe the terminalia of this species also. I wish to thank Dr. Rodhain and Mr. Pfaff for these gifts.

SALIENT DIAGNOSTIC CHARACTERS OF THE TERMINALIA. The interest attached to the segmentation of the abdomen in this genus is that in the male of both species studied there is an additional plate distal to and above sternum 6 (which is also complete), which I take to be sternum 7; the membrane which normally stretches from sternum 6 to the ninth tergo-sternum, and which in all the higher Diptera forms a pocket for the phallosome, in these two species stretches from sternum 7 to the ninth tergo-sternum. This plate is shown in fig. 3. I have not seen this additional plate in any of the other higher Diptera so far examined. The segmentation of the female abdomen too is complete, all the terga and sterna being represented and well developed.

***Cobboldia elephantis* Steel.** ♂. Fig. 1. Sternum 5 is a relatively long wide plate, shaped as shown in fig. 1, *b*. The ninth tergo-sternum (fig. 1, *c*) is long and narrow, with a deep U-shaped incision. The anal cerci (fig. 1, *d*) are completely fused, and together form a wide, rather flat plate terminating in a wide square-cut end. The proximal segment of the ninth coxite is a short wide plate, joined to its fellow in the middle and hinged to the postero-lateral edge of the distal segment. The distal segment (fig. 1, *d*) is a long, bent, rounded, finger-like plate, forming with its fellow a lateral clasper. The phallosome

(fig. 1, *a*) is relatively long, the distal part consisting of a pair of ventral plates, to each of which is attached on the dorsal side a heavily spined flap, the two flaps enclosing between them a wide chitinous tube; on the dorsal side there is a strong spined plate attached by membrane to the end of each of the ventral plates. The basal part of the phallosome is long and wide, the posterior process,

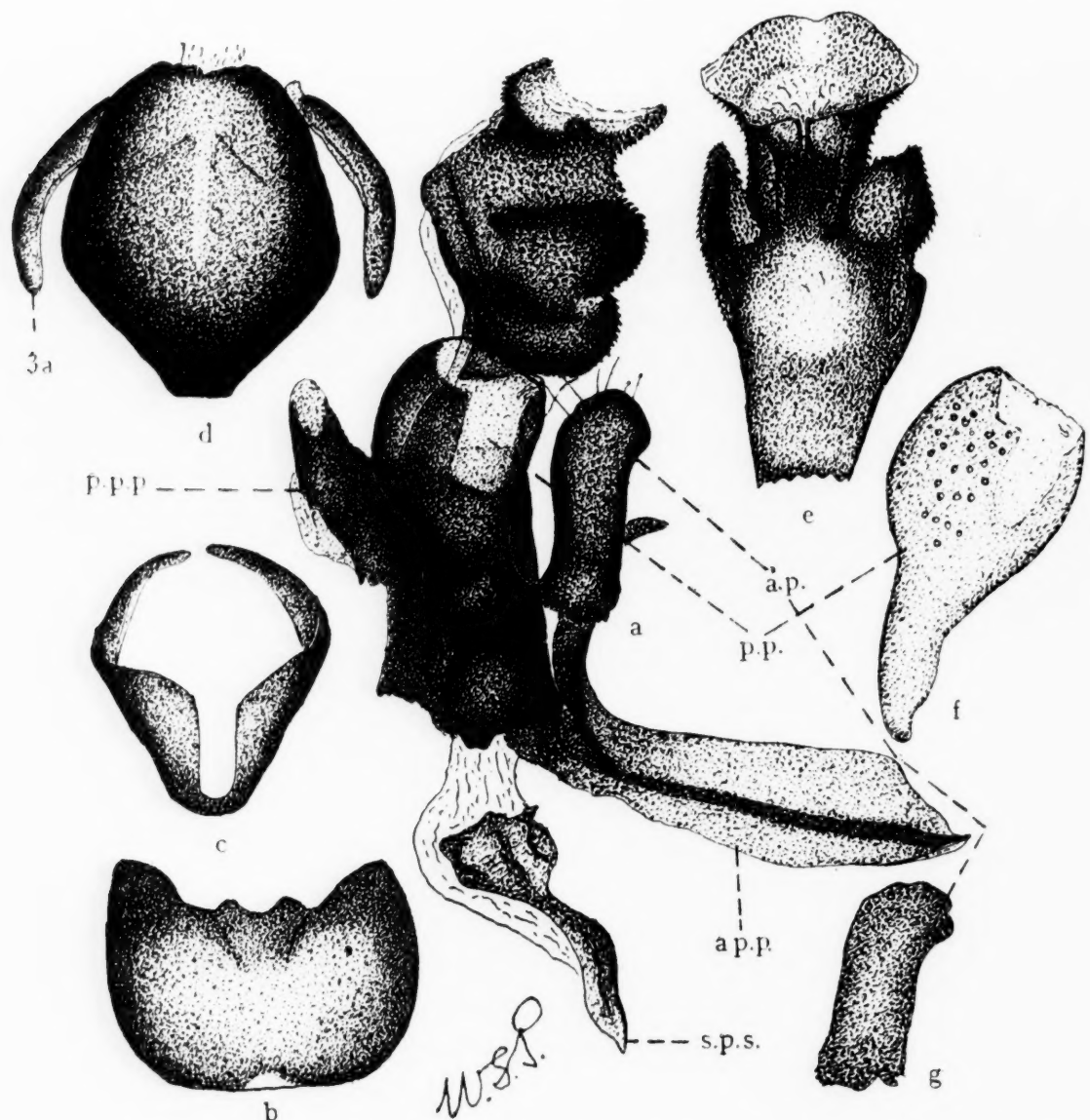


FIG. 1. *a*.—Phallosome and one paramere of *C. elephantis* in side view; *a.p.*—Anterior paramere; *ap.p.*—Apodeme of phallosome; *p.p.*—Posterior paramere; *p.p.p.*—Posterior process of phallosome; *s.p.s.*—Sperm pump sclerite; *b*.—Fifth sternum; *c*.—Ninth tergo-sternum; *d*.—Anal cerci and distal segments of ninth coxites; *e*.—Ventral view of end of phallosome; *f*.—Right posterior paramere; *g*.—Raised part of anterior paramere.

arising at an acute angle from the base, is long and rounded. The anterior part of the paramere (fig. 1, *a*) is a raised plate rounded distally, with several short spine-like hairs and a long ventral prolongation; the posterior part (fig. 1, *a*, *f*) is a large plate, somewhat flattened laterally, expanding distally, and armed with many short spine-like hairs on the outer surface.

♀. Fig. 2. The ovipositor is long, and the various terga and sterna are not only present but well developed. Sterna 6, 7 and 9 have a hairy chitinous area on each side at the distal end, those of sterna 6 and 7 being long and narrow, those of sterna 6 and 7 being long and narrow,

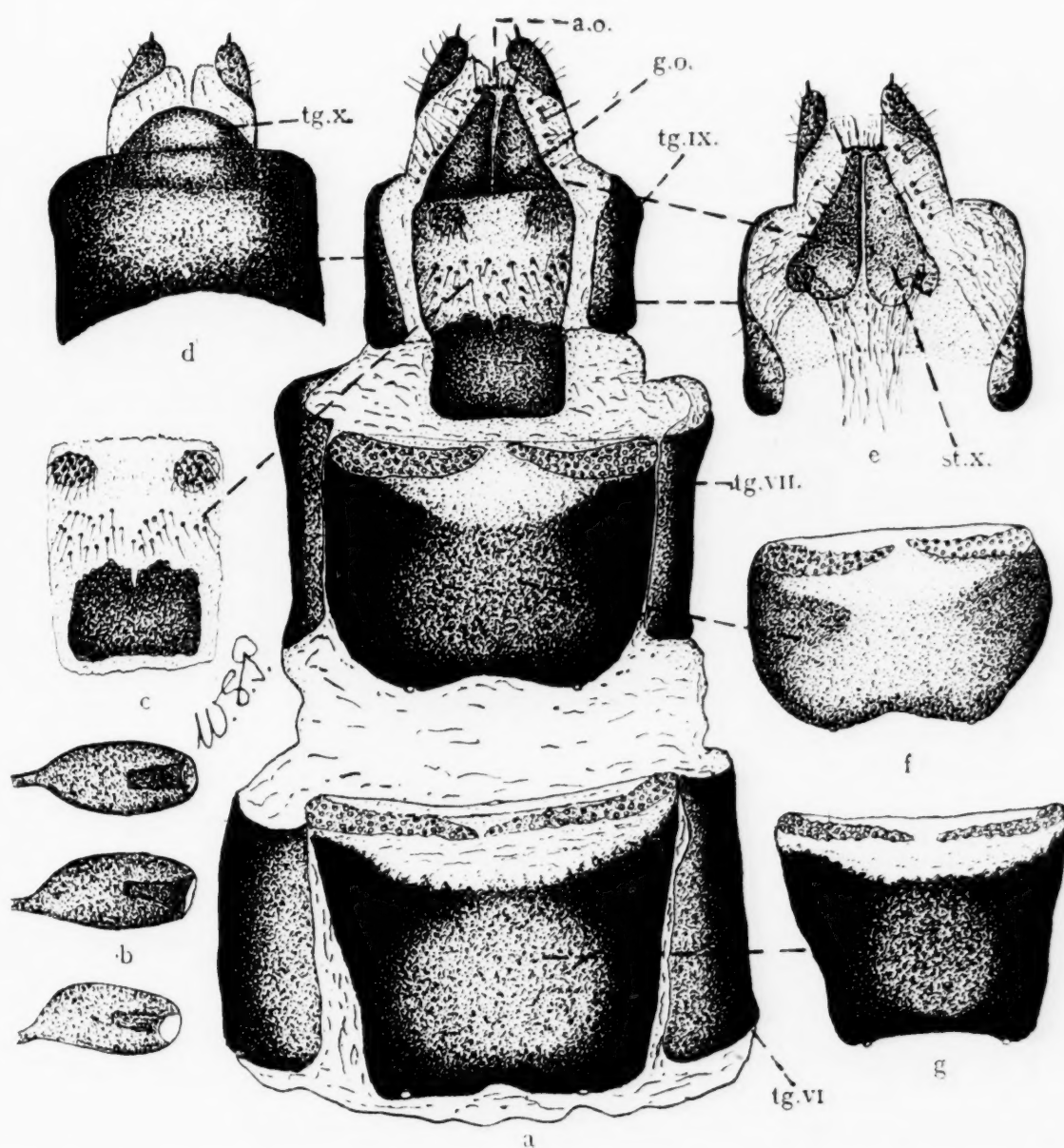


FIG. 2. *a.*—Ventral view of ovipositor of *C. elephantis*; *a.o.*—Anal opening; *g.o.*—Genital opening; *tg.vi*, *tg.vii*, *tg.ix*.—Sixth, seventh, ninth terga; *b.*—Spermathecae; *c.*—Ninth sternum; *d.*—Dorsal view of end of ovipositor; *tg.x.*—Tenth tergum; *e.*—Ventral view of end of ovipositor; *st.x.*—Tenth sternum; *f.*—Seventh sternum; *g.*—Sixth sternum.

while those of sternum 9 are small and round. Sternum 10 consists of a pair of triangular-shaped plates joined together and armed with short hairs; tergum 10 is a small rounded plate. The spermathecae (fig. 1, *b*) are large and have a characteristic funnel-shaped depression at the rounded end.

Cobboldia chrysidiformis Rodhain and Bequaert. ♂. Figs. 3; 4. Sternum 5 (fig. 4, *b*) is a short wide plate, with rather long lateral arms with straight ends, and a bluntly pointed process on each side of the middle line. The ninth tergosternum (fig. 4, *c*) is very similar to that of *elephantis*. The anal cerci (fig. 4, *d*) are also structurally similar to those of *elephantis*, and are fused to form a flat plate, the free end being longer and wider. The ninth coxite (figs. 3; 4, *d*) is also very similar to that of *elephantis*, the distal segment forming a long, round, finger-like plate. The phallosome (fig. 4, *a*) is structurally similar to that of *elephantis*, and in the single specimen examined the posterior membrane on the ventral side at the distal end is drawn in, giving the ventral edge a pointed

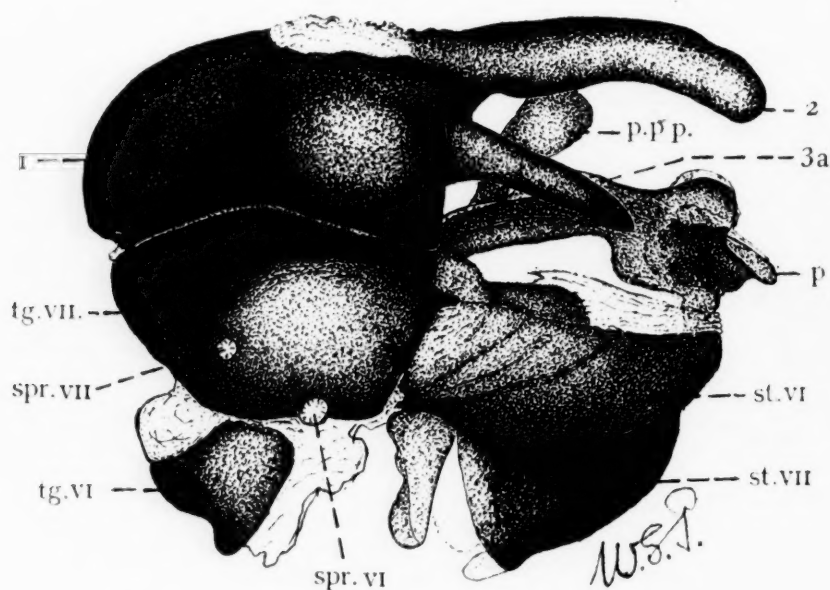


FIG. 3. Sixth, seventh and tenth segments of ♂ *C. chrysidiformis* in side view; 1.—Tenth tergum; 2.—Anal cerci; 3a.—Distal segment of ninth coxite; p.—Phallosome; p.p.p.—Posterior process of phallosome; st.vi, st.vii.—Sixth, seventh sterna; spr.vi, spr.vii.—Sixth, seventh spiracle; tg.vi, tg.vii.—Sixth, seventh terga.

appearance in side view. The anterior part of the paramere (fig. 4, *a*) is a small plate rounded at the end and bearing a number of short, stiff, spine-like hairs; it also has a long ventral prolongation; the posterior part (fig. 4, *a*) is an upstanding plate with a short bluntly pointed end, and has numerous short hairs on the outer surface.

♀. Fig. 5. The ovipositor of *chrysidiformis* closely resembles that of *elephantis*, as will be noted by comparing figs. 2 and 5. The strongly chitinized area at the posterior end of sternum 9 is shaped like the wings of a butterfly, while the similar area on sternum 9 of *elephantis* is almost square; there are other minor differences in the shape of the chitinized areas on the sterna.

SYSTEMATIC POSITION OF *COBBOLDIA*. The comparative study of the terminalia of these two species of *Cobboldia* has demonstrated the fact that they are closely related to each other. It will be remembered that *C. elephantis* and *C. loxodontis* are both placed in the genus *Cobboldia*, whereas *C. chrysidiformis* is placed in a separate genus, *Rodhainomyia*. Without studying the terminalia of *loxodontis*, I hesitate to express an opinion on this point of grouping the

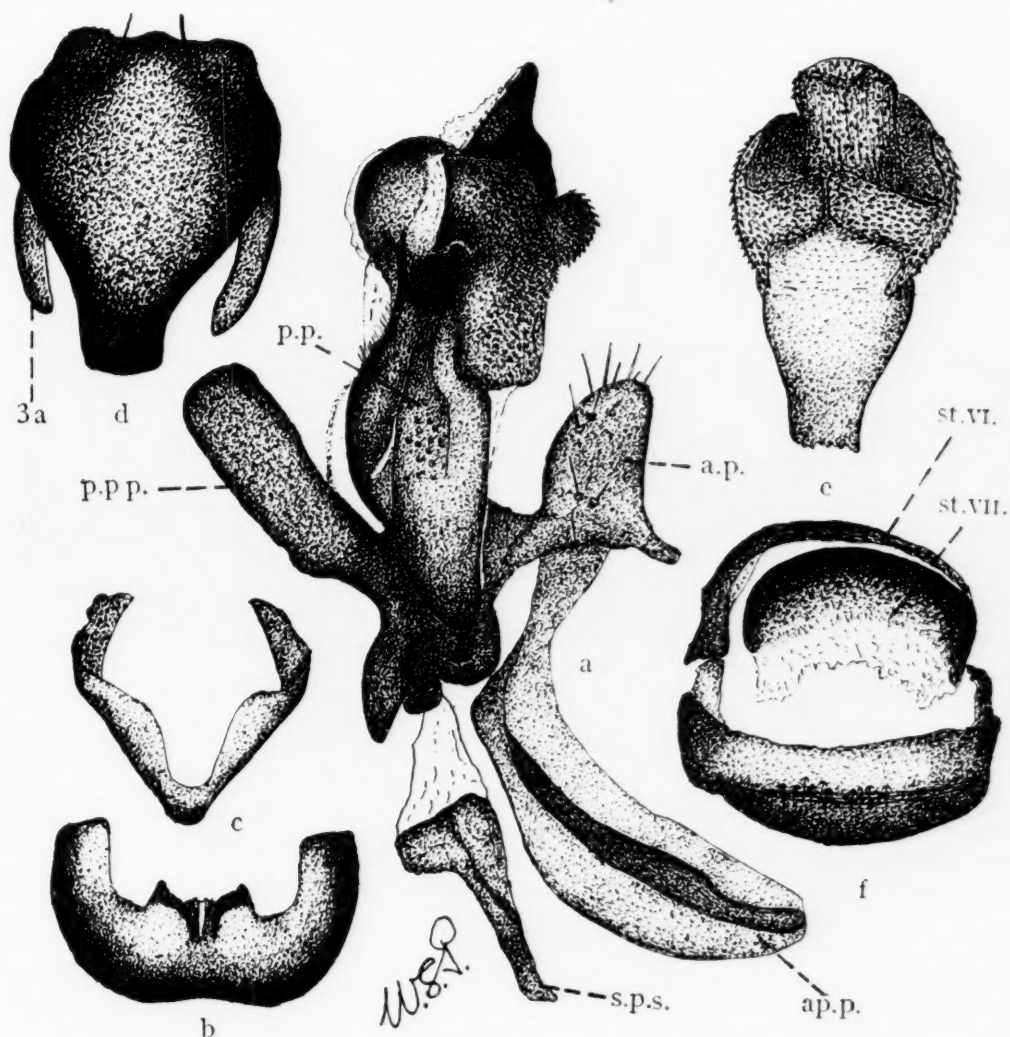


FIG. 4. a.—Phallosome and one paramere of *C. chrysidiformis* in side view; lettering as in fig. 1, a; b.—Fifth sternum; c.—Ninth tergo-sternum; d.—Anal cerci and distal segments of ninth coxites; e.—Ventral view of end of phallosome; f.—Ventral view of sixth and seventh sterna.

species, and will leave it to another occasion when I have had an opportunity of examining the terminalia of *loxodontis*. At present I know of no other higher Dipteran with similar terminalia to those of these two species of *Cobboldia*, and I think it best, therefore, to place these flies in a subfamily by themselves, the Cobboldiinae. As I know of no other higher Diptera with similar terminalia

I am unable to say to which family they belong ; I certainly think that it is premature to place them in the Tachinidae.

Ruttenia loxodontis Rodhain. The egg, larva and adult of this interesting fly have been described by Rodhain (1924, 1927) ; the larva is a subcutaneous parasite of the African elephant. Dr. Rodhain very kindly gave me two females

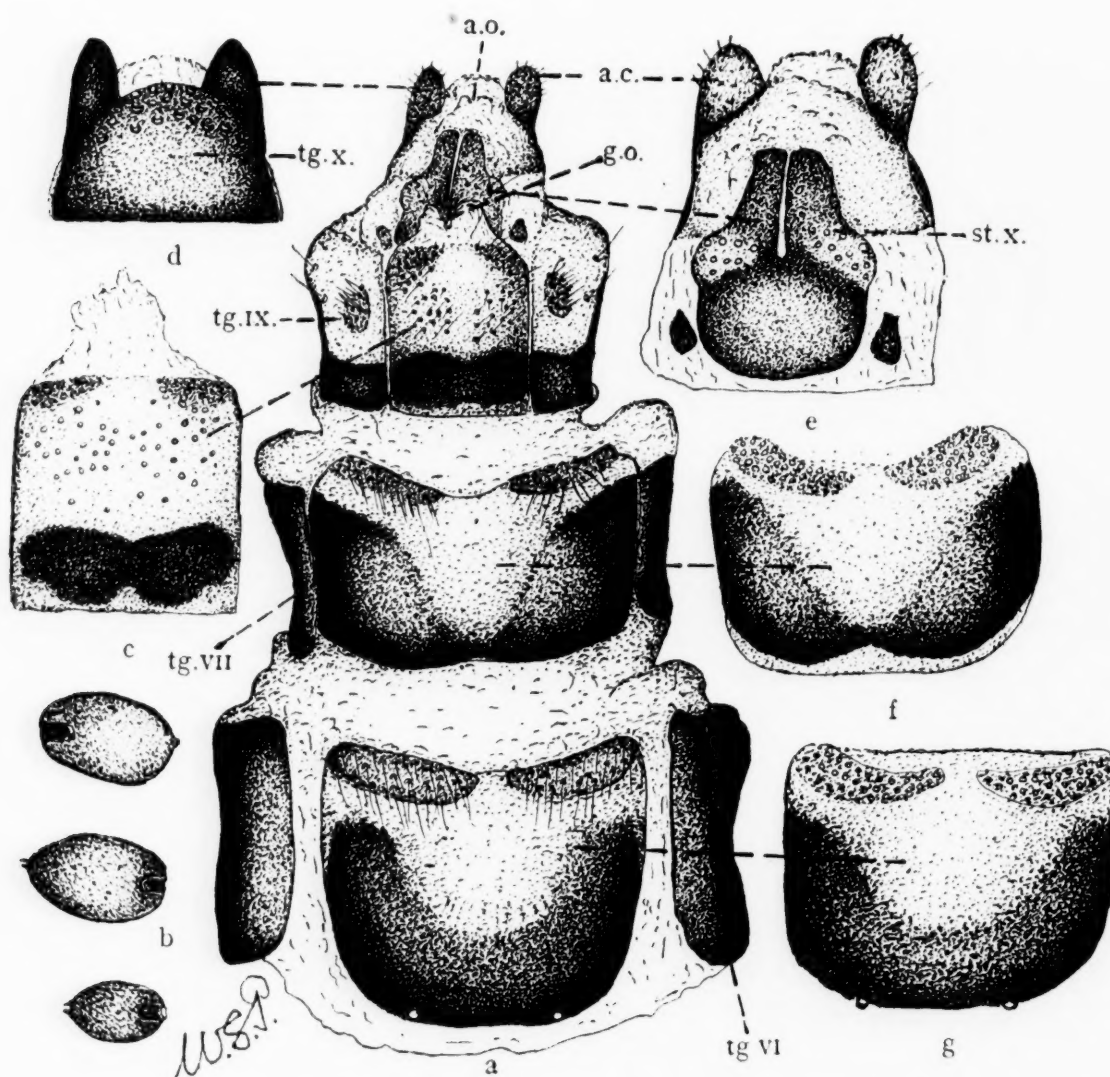


FIG. 5. *a*.—Ventral view of ovipositor of *C. chrysidiformis* ; lettering as in fig. 2, *a* ; *b*.—Spermathecae ; *c*.—Ninth sternum ; *d*.—Dorsal view of end of ovipositor ; *e*.—Ventral view of same ; lettering as in fig. 2, *e* ; *f*.—Seventh sternum ; *g*.—Sixth sternum.

and the abdomen of a male, and I am therefore able to illustrate and note the terminalia. I wish to thank Dr. Rodhain for his generous gift.

SALIENT DIAGNOSTIC CHARACTERS OF TERMINALIA. ♂. Fig. 6. Sternum 5 (fig. 6, *b*) is a large wide plate, the posterior end narrow and the lateral lobes round and wide, the whole being very suggestive of the corresponding plate

of *Hypoderma*. The ninth tergo-sternum (fig. 6, *c*) is a simple, rather small plate, the posterior arms narrow and pointed. The distal segment of the ninth coxite (fig. 6, *e, f*) is a short broad plate, rounded at the end and forming with its fellow a lateral clasper; the proximal segment is a short plate joined to its fellow in the middle line. The anal cerci (fig. 6, *e, f*) are fused, and together form a small bluntly pointed process lying in the middle line between the distal

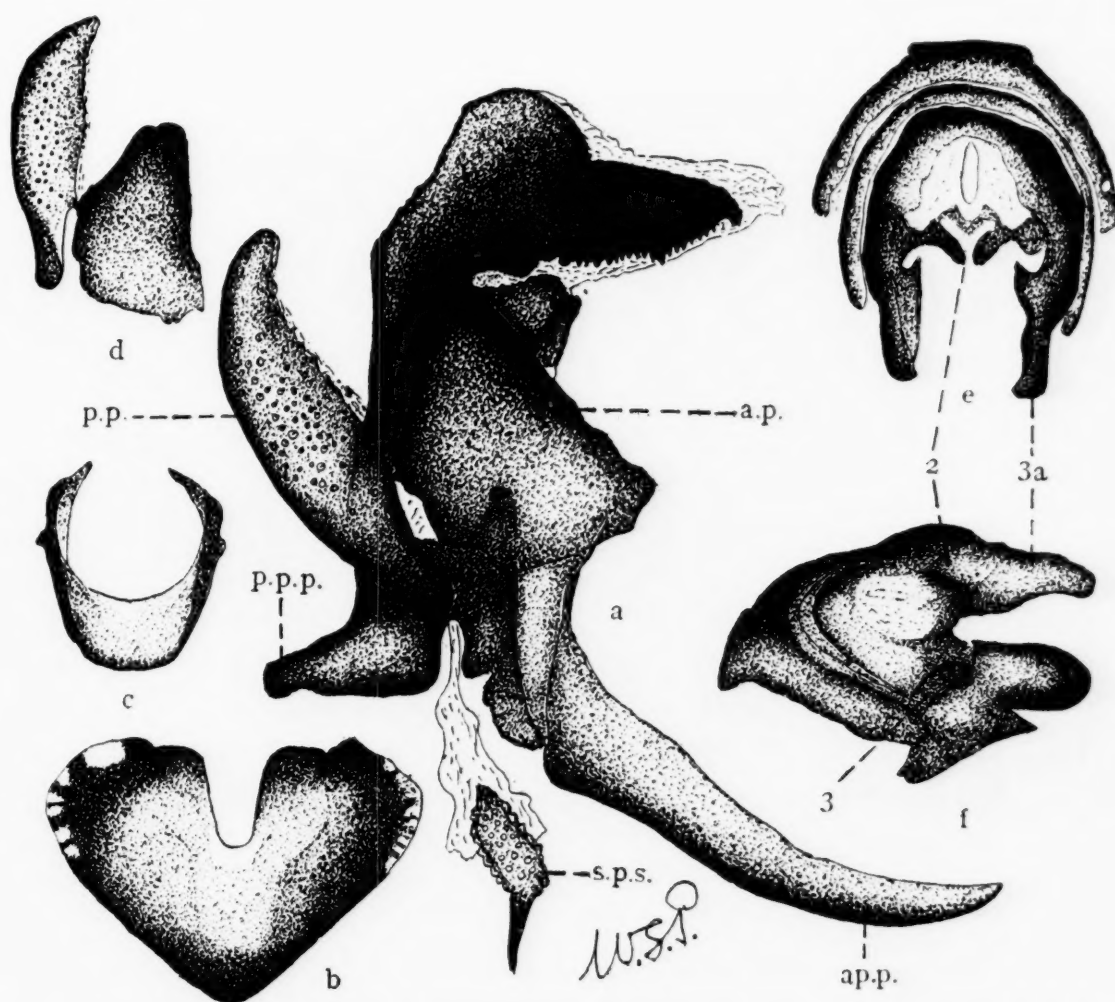


FIG. 6. *a*.—Phallosome and one paramere of *R. loxodontis* in side view; lettering as in fig. 1, *a*; *b*.—Fifth sternum; *c*.—Ninth tergo-sternum; *d*.—Two parts of right paramere; *e*.—Ventral view of sixth, seventh and tenth segments, showing anal cerci and distal segments of ninth coxites; 2.—Anal cerci; 3*a*.—Distal segment of ninth coxite; *f*.—Lateral view of same, showing anal cerci and ninth coxite; 3.—Proximal segment of ninth coxite.

segments of the ninth coxites; in fig. 6, *e*, the two parts of the anal cerci are shown separated. The phallosome (fig. 6, *a*) is short, the distal part consisting ventrally of two broad plate-like struts expanded at their ends and having on the outer side many short, pointed, appressed spines; projecting from between the two ends on the ventral side there is a broad, densely chitinized plate, ending in a blunt point, with a row of short teeth-like serrations along its dorsal border;

it is surrounded by membrane which carries the opening of the ejaculatory duct ; the basal part of the phallosome is short and has a stout posterior process projecting ventrally. The anterior part of the paramere (fig. 6, *a, d*) is a wide plate, flattened from side to side, with a rounded extremity ; the posterior part (fig. 6, *a, d*) is a long upstanding plate with numerous fine spine-like hairs on its outer surface.

♀. Fig. 7. The ovipositor is relatively short ; the terga and sterna are all present and well developed. Tergum and sternum 7 are fused to form a

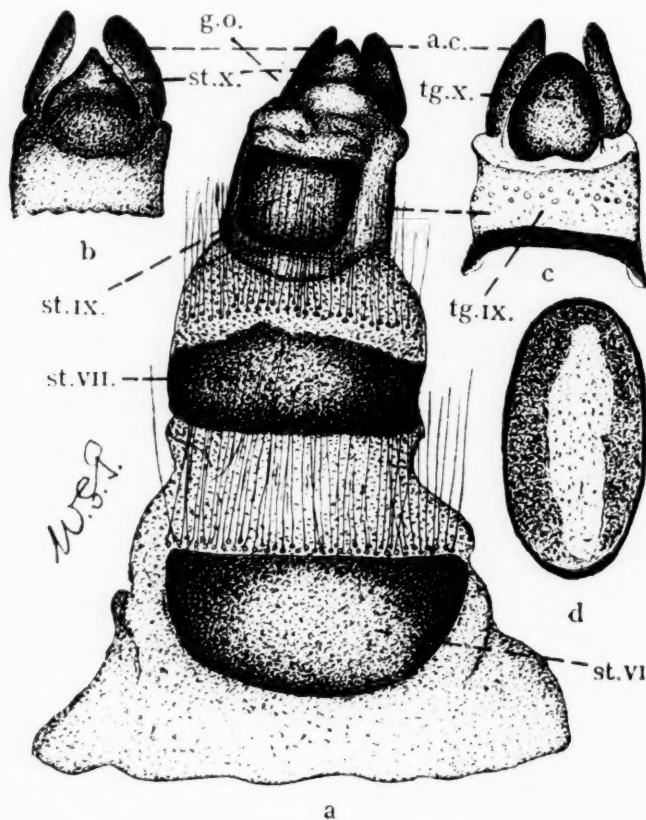


FIG. 7. *a*.—Ventral view of ovipositor of *R. loxodontis* ; lettering as in fig. 2, *a* ; *b*.—Ventral view of end of ovipositor ; *c*.—Dorsal view of same ; lettering as in fig. 2, *d* ; *d*.—Egg.

chitinous tube ; sternum 9 is rectangular in shape, while sternum 10 is triangular-shaped. Tergum 10 is oval, and the anal cerci are long. The egg (fig. 7, *d*), which Rodhain has illustrated, is oval and has a thick shell and a roughened area all round the margin on the ventral side. It is presumed (though this is not known for certain) that the egg is laid directly on the skin, as there is no flange for attachment to a hair.

SYSTEMATIC POSITION OF *RUTTENIA LOXODONTIS*. A comparative study of the ♂ terminalia of *R. loxodontis* with those of species of *Hypoderma* suggests some relationships. In *Hypoderma* the struts of the phallosome are narrow and

pointed, while those of *Ruttenia* are wide and expanded at the end ; in *H. tarandi* and *H. diana* there is a suggestion of a median chitinous plate between the ends of the struts. The small fused anal cerci, the distal segments of the ninth coxites, the parameres and especially the fifth sternum are all suggestive of those of *Hypoderma*. The ovipositor is adapted to lay the egg on the skin (the structure of the egg suggesting this), and therefore the end is not modified, as in *Hypoderma*, for laying the egg on a hair. I consider, then, that *Ruttenia* is probably allied to *Hypoderma* and is best placed in the subfamily Hypodermatinae.

It is interesting to note here that subcutaneous parasitism due to the larvae of a higher Dipteron has originated more than once in the case of the elephant, for we have two species with this habit in Africa, *R. loxodontis*, whose larva lives in the skin of the body, and *Neocuterebra squamosa*, whose larva lives in the sole of the foot. I have not had an opportunity of examining the terminalia of the latter and can express no opinion as to its relationships. In the case of the Indian elephant, on the other hand, the larva of a species of *Cordylobia*, *C. indica*, parasitizes the skin of the body.

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STUDIES ON THE HIGHER DIPTERA OF MEDICAL AND VETERINARY IMPORTANCE

ILLUSTRATIONS OF THE TERMINALIA OF SOME SPECIES OF *GASTEROPHILUS*

BY

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(Received for publication 24 June, 1937)

In an earlier paper (1935) I described the segmentation of the abdomen and the terminalia of *Gasterophilus intestinalis* de Geer. Since then Dr. Sczilady has kindly given me specimens of *G. pecorum*, and Dr. Salem a male of *G. flavipes* Olivier (*albescens* Pleske), and in the present paper I shall describe the terminalia of these two species, as well as those of *G. nasalis* and *G. haemorrhoidalis*. As the illustrations speak for themselves, I shall only briefly note the important diagnostic characters in each case. I hope that these illustrations will later help in clearing up the identity of those species of *Gasterophilus* whose larvae are parasitic in the stomach of the zebra, for I feel certain that the status of these species will not finally be settled until their terminalia have been studied.

***Gasterophilus haemorrhoidalis* L.** SALIENT DIAGNOSTIC CHARACTERS OF TERMINALIA. ♂. Fig. 1. The distal segment of the ninth coxite (fig. 1, *a, h*) is a long rounded plate, ending in a short blunt point directed towards the middle line; it has numerous long hairs along its inner and ventral borders; the proximal segment is a short broad plate, joined to its fellow by membrane and articulating with the ninth tergo-sternum. It has on its outer edge a raised, hairy, chitinous, pad-like plate (fig. 1, *f*); I believe that this plate, with its fellow, helps to hold the end of the ovipositor when it is inserted into the genital atrium of the male. The distal end of the ninth coxite is illustrated in side view in fig. 1, *h*. The anal cerci (fig. 1, *a, h*) are small, convex, somewhat elongated plates, one on each side of the anal opening; they can clearly take only a minor part in copulation. The ninth tergo-sternum (fig. 1, *b*) is a strong, wide, square plate structurally similar to that of *intestinalis*; the posterior processes are bent round so as almost to meet, and are dilated at the end and hollowed out on the inner face, which is armed with fine appressed apines. The phallosome (fig. 1, *c, d*) is a very short, rectangular, chitinous tube when seen in side view, the anterodorsal face excavated and membranous, the ventral wall strengthened to form a strong, rod-like, chitinous support (strut). The apodeme (fig. 1, *d*) is a strong plate, flattened laterally and firmly attached in the middle line to the ventral side of the proximal end of the ninth tergo-sternum by a strong wing-like expansion on each side; the distal end is loosely attached to the phallosome by membrane. The anterior paramere (fig. 1, *d, g*) is a hairy convex plate bearing long hairs;

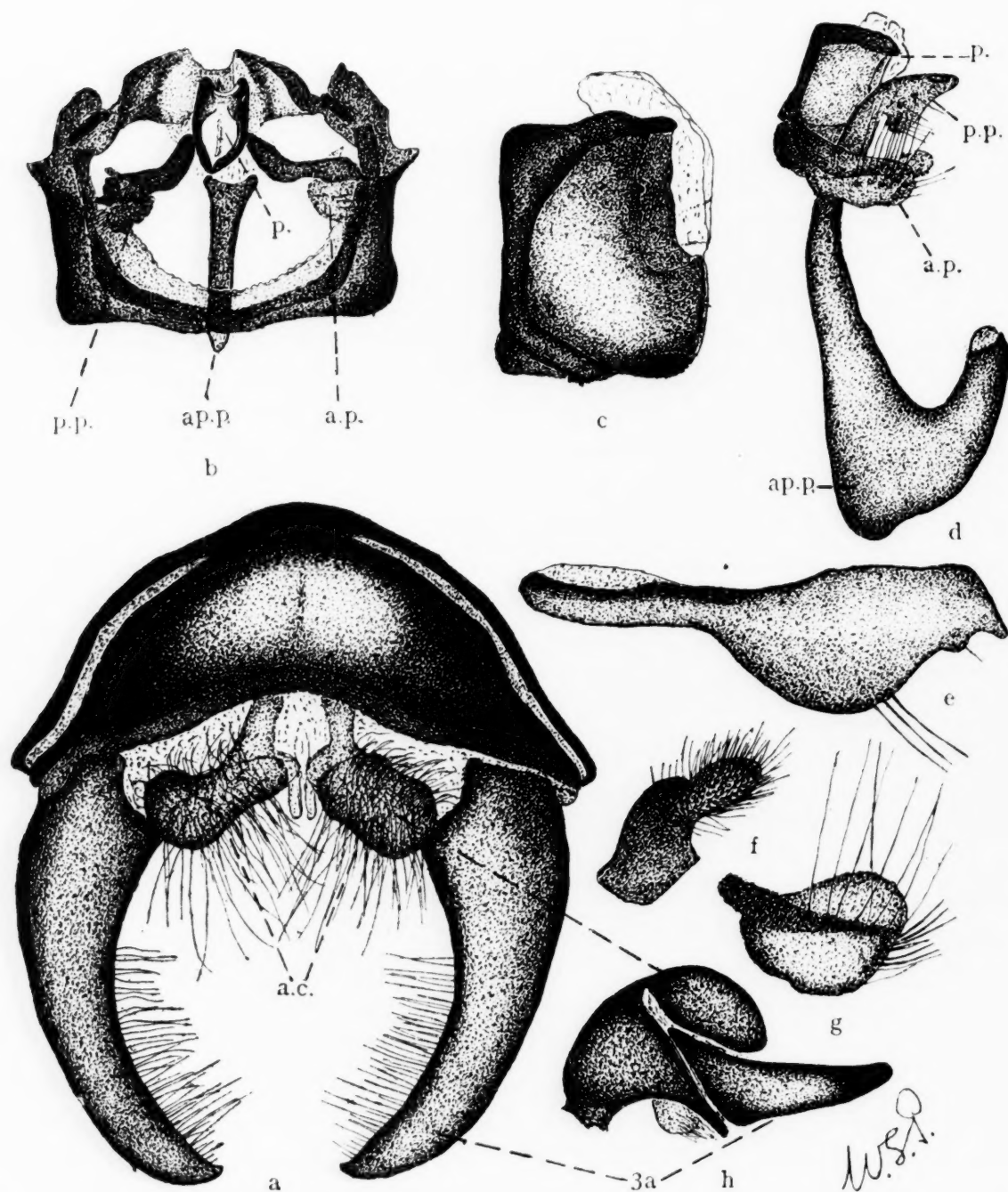


FIG. 1. *a.*—Ventral view of tenth tergum of ♂ *G. haemorrhoidalis*, showing anal cerci and distal segments of ninth coxites; *a.c.*—Anal cerci; *3a.*—Distal segment of ninth coxite; *b.*—Antero-dorsal view of ninth tergo-sternum, showing phallosome, apodeme and parameres; *a.p.*—Anterior paramere; *ap.p.*—Apodeme of phallosome; *p.*—Phallosome; *p.p.*—Posterior paramere; *c.*—Lateral view of phallosome; *d.*—Phallosome and one paramere in side view; lettering as in *b*; *e.*—Lateral view of right posterior paramere; *f.*—Part of proximal segment of ninth coxite, showing hairy pad-like plate; *g.*—Lateral view of right anterior paramere; *h.*—Lateral view of tenth segment, showing anal cercus and distal segment of ninth coxite.

the posterior part (fig. 1, *d, e*) is a long rod-like plate, and in side view is seen to end in a hook-like point.

♀. Fig. 2. The ovipositor is structurally similar to that of *intestinalis*. Tergum and sternum 7 are fused to form a strong chitinous tube. The ninth sternum is expanded at the end. As in *intestinalis*, there are only two spermathecae (fig. 2, *c*).

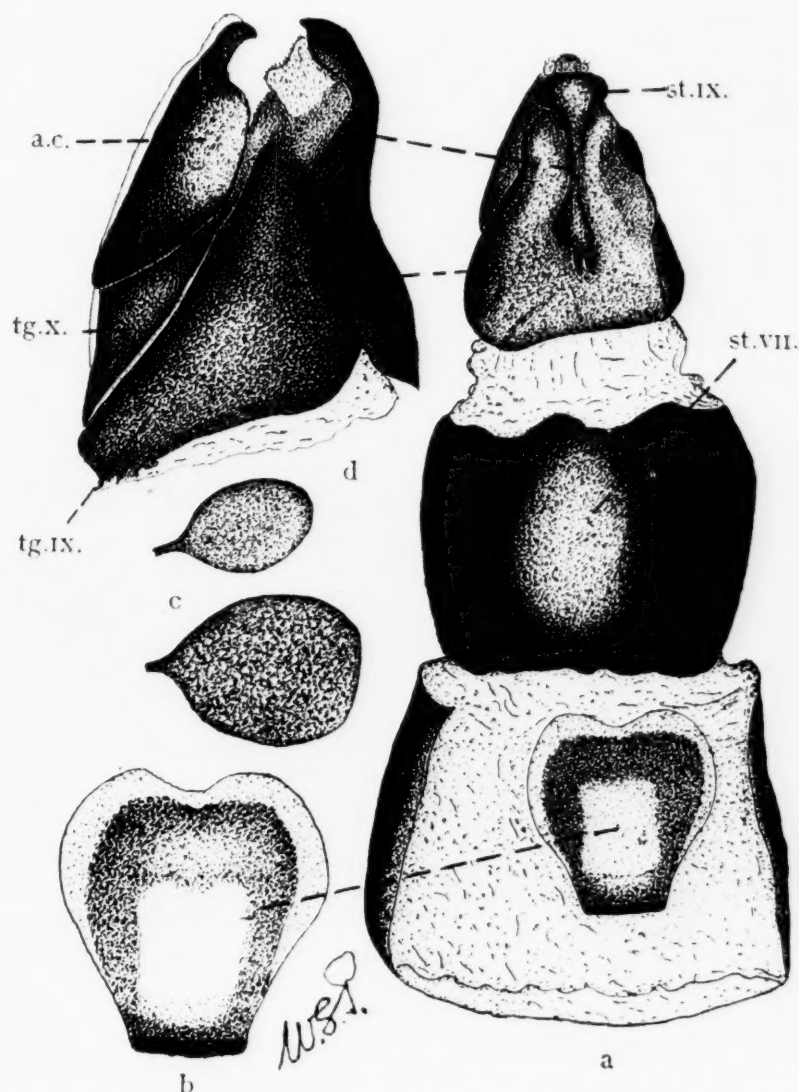


FIG. 2. *a*.—Ventral view of ovipositor of *G. haemorrhoidalis*; *st.vii, st.ix*.—Seventh, ninth sterna; *b*.—Sixth sternum; *c*.—Spermathecae; *d*.—Lateral view of end of ovipositor; *a.c.*—Anal cercus; *tg.ix, tg.x*.—Ninth, tenth terga.

Gasterophilus flavipes Olivier (*albescens* Pleske). SALIENT DIAGNOSTIC CHARACTERS OF TERMINALIA. ♂. Fig. 3. The terminalia of this species are very similar to those of *haemorrhoidalis*. The distal segment of the ninth coxite (fig. 3, *a, f*) is a long rounded plate ending in a blunt point which is turned towards its fellow; it has many long hairs on its inner surface. The proximal segment (fig. 3, *d*) also has a pad-like, convex, hairy plate attached to its outer

edge as in *haemorrhoidalis*, and probably has the same function. The anal cerci (fig. 3, *a*) are small rather rectangular plates (in ventral view) and lie on each side of the anal opening. The ninth tergo-sternum (fig. 3, *b*) is very similar to that of *haemorrhoidalis*. The phallosome (fig. 3, *c*) is small and rounded, and has a short, rod-like, supporting plate on each side ventrally; the apodeme

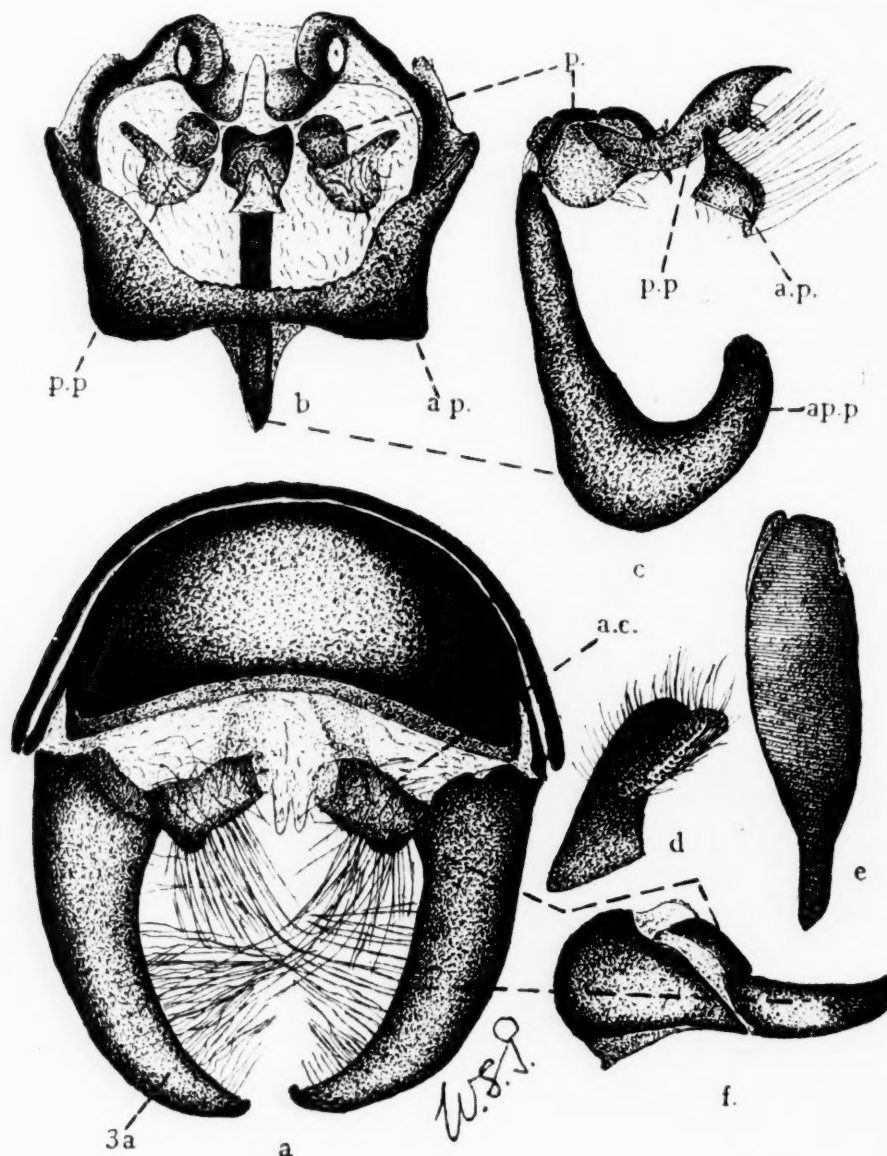


FIG. 3. *a*.—Ventral view of tenth tergum of *G. flavipes*, showing anal cerci and distal segments of ninth coxites; lettering as in fig. 1, *a*; *b*.—Antero-dorsal view of ninth tergo-sternum; lettering as in fig. 1, *b*; *c*.—Phallosome and one paramere in side view; lettering as in fig. 1, *b*; *d*.—Part of proximal segment of ninth coxite, showing pad-like hairy plate; *e*.—Egg; *f*.—Lateral view of tenth tergum, showing anal cercus and distal segment of ninth coxite.

(fig. 3, *c*) is a strong flattened plate (side to side), and is attached to the ninth tergo-sternum by a flange on each side. The anterior part of the paramere (fig. 3, *b, c*) is a small, irregularly shaped plate, convex dorsally and covered with long hairs; the posterior part (fig. 3, *b, c*) in side view is seen to be a bent rod-like plate ending in a sharp point. I have not seen the female of this species

and am therefore unable to illustrate the terminalia. An egg believed to belong to this species was found lying in the ♂ genital atrium; it will be noted from the illustration (fig. 3, *e*) that it is very similar to that of *haemorrhoidalis*.

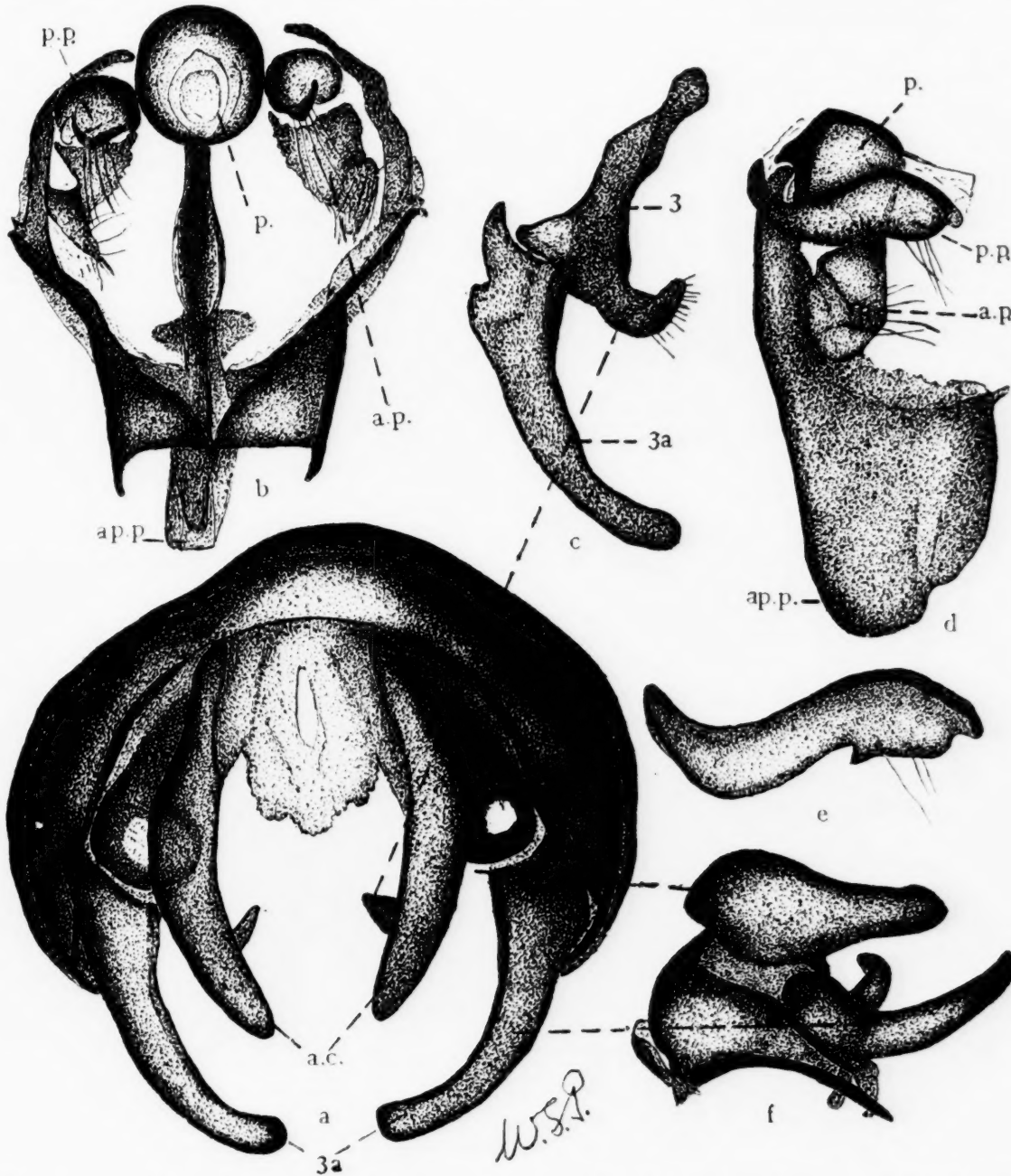


FIG. 4. *a*.—Ventral view of tenth tergum of *G. nasalis*, showing anal cerci and distal segments of ninth coxites; lettering as in fig. 1, *a*; *b*.—Antero-dorsal view of ninth tergo-sternum, showing phallosome, etc.; lettering as in fig. 1, *b*; *c*.—Distal segment of left ninth coxite from ventral side, showing finger-like hairy process on inner side; 3.—Proximal segment; 3*a*.—Distal segment; *d*.—Phallosome and one paramere in side view; lettering as in fig. 1, *b*; *e*.—Lateral view of right posterior paramere; *f*.—Lateral view of tenth tergum, to show anal cercus and distal segment of ninth coxite.

***Gasterophilus nasalis* L. SALIENT DIAGNOSTIC CHARACTERS OF TERMINALIA.**

♂. Fig. 4. The distal segment of the ninth coxite (fig. 4, *a*, *b*) is a long, rounded, narrow plate with a straight blunt end directed inwards towards its fellow;

it has only a few hairs on the inner surface. The proximal segment (fig. 4, *c*) is a stout plate with a projection about the middle of the outer side, by which it articulates with the distal segment; it has on its inner side a long, hairy, finger-like process directed inwards and downwards. The anal cerci (fig. 4, *a, b*) are also long, rounded, blunt-pointed plates, simulating somewhat the distal

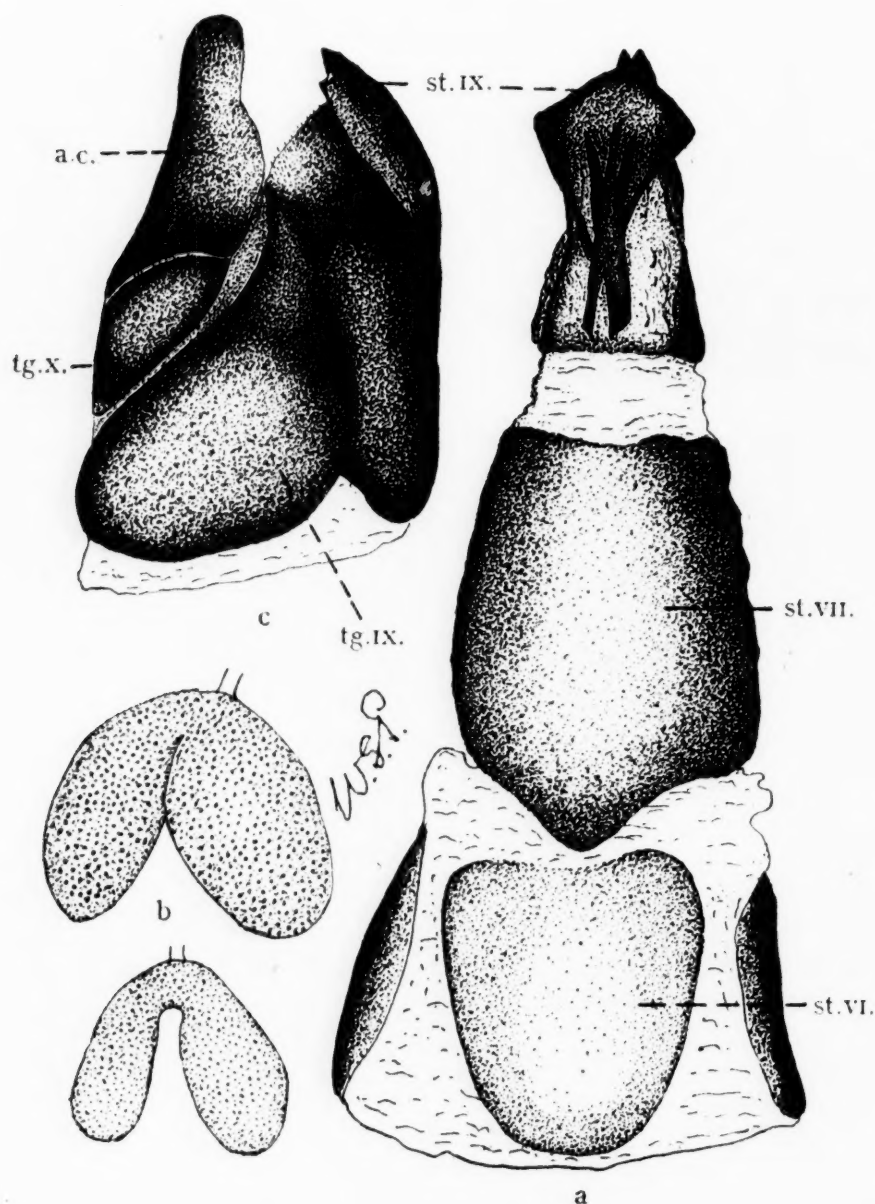


FIG. 5. *a*.—Ventral view of ovipositor of *G. nasalis*; lettering as in fig. 2, *a*; *b*.—Spermathecae; *c*.—Lateral view of end of ovipositor; lettering as in fig. 2, *d*.

segment of the ninth coxite. The ninth tergo-sternum (fig. 4, *b*) is wide, and similar to that of the other species; the posterior processes are narrow and do not meet, and in the interval between them the phallosome lies. The phallosome (fig. 4, *d*) is very short and somewhat rectangular in shape, the opening of the ejaculatory duct being on a membranous part; the apodeme (fig. 4, *d*) is very

similar to that of the other species. The anterior paramere (fig. 4, *b, d*) is an elongated, convex, hairy plate; the posterior part (fig. 4, *b, d*) is a long bent plate (in side view, fig. 4, *e*) and ends in a blunt point.

♀. Fig. 5. The ovipositor is structurally similar to that of *intestinalis* and *haemorrhoidalis*, the only important difference being that the end of the ninth sternum is markedly expanded. The spermathecae (fig. 5, *b*) are looped, and one is larger than the other.

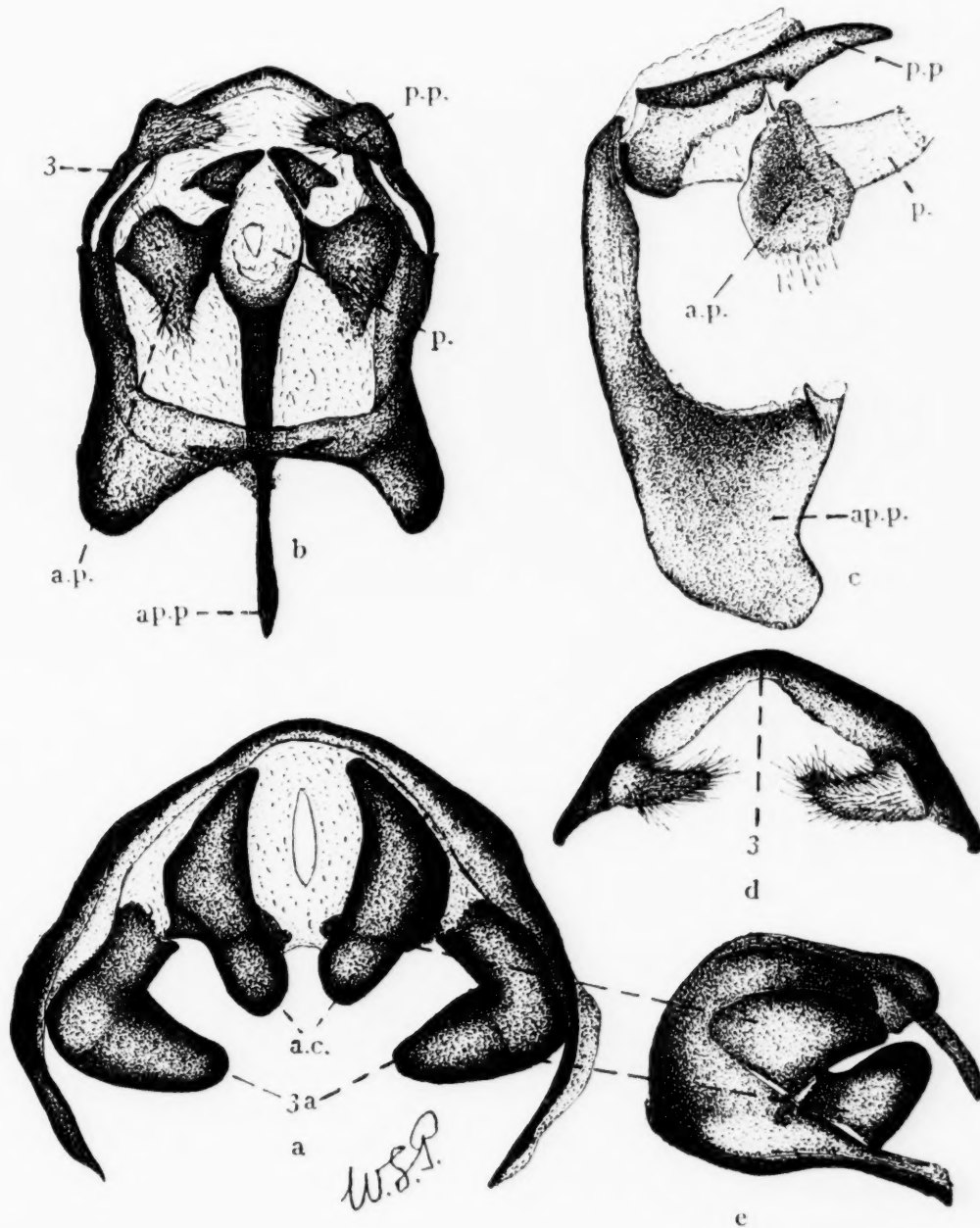


FIG. 6. *a*.—Ventral view of tenth tergum of ♂ *G. pecorum*, showing anal cerci and distal segments of ninth coxites; lettering as in fig. 1, *a*; *b*.—Antero-dorsal view of ninth tergo-sternum, showing phallosome, etc.; lettering as in fig. 1, *b*; *c*.—Phallosome and one paramere in side view; lettering as in fig. 1, *b*; *d*.—The fused proximal segments of ninth coxites, showing finger-like hairy process on each; *e*.—Lateral view of tenth segment, showing anal cercus and distal segment of ninth coxite.

it has only a few hairs on the inner surface. The proximal segment (fig. 4, *c*) is a stout plate with a projection about the middle of the outer side, by which it articulates with the distal segment; it has on its inner side a long, hairy, finger-like process directed inwards and downwards. The anal cerci (fig. 4, *a*, *b*) are also long, rounded, blunt-pointed plates, simulating somewhat the distal

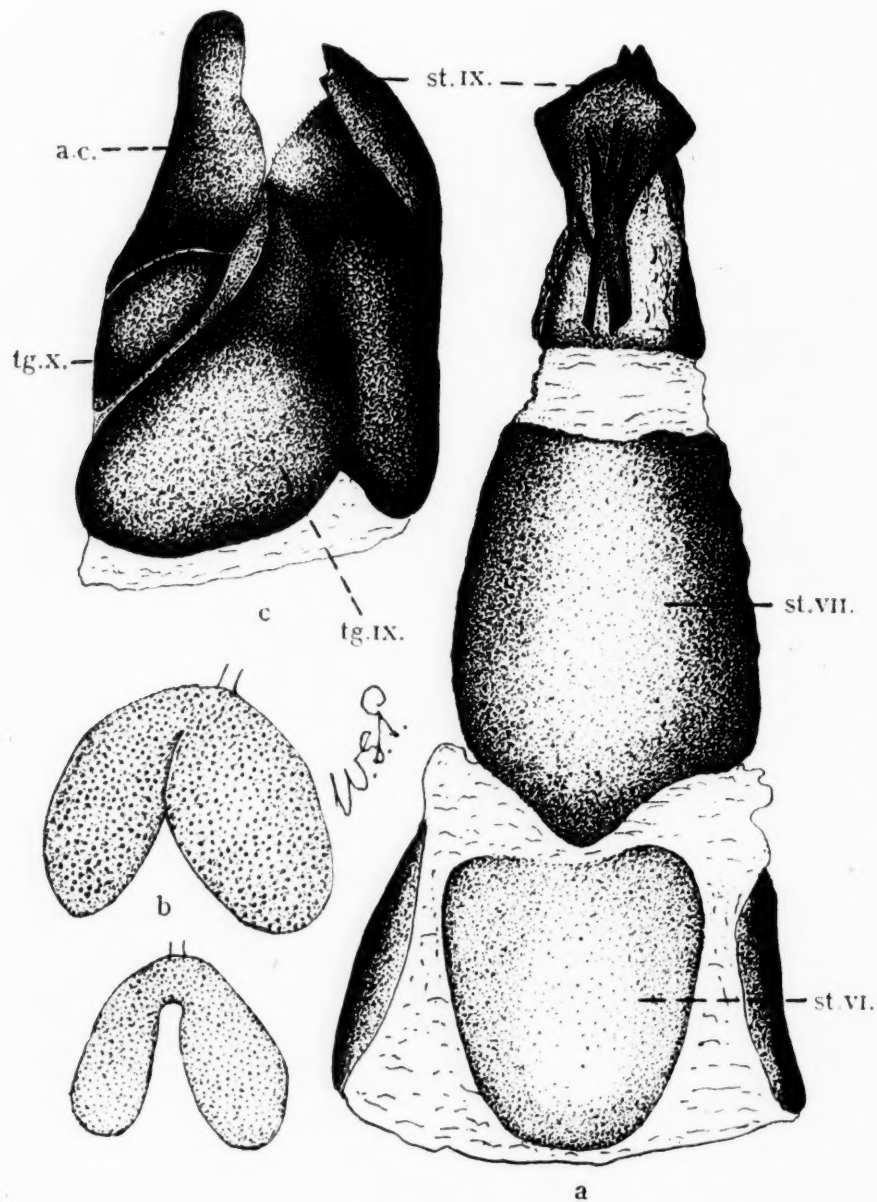


FIG. 5. *a*.—Ventral view of ovipositor of *G. nasalis*; lettering as in fig. 2, *a*; *b*.—Spermathecae; *c*.—Lateral view of end of ovipositor; lettering as in fig. 2, *d*.

segment of the ninth coxite. The ninth tergo-sternum (fig. 4, *b*) is wide, and similar to that of the other species; the posterior processes are narrow and do not meet, and in the interval between them the phallosome lies. The phallosome (fig. 4, *d*) is very short and somewhat rectangular in shape, the opening of the ejaculatory duct being on a membranous part; the apodeme (fig. 4, *d*) is very

similar to that of the other species. The anterior paramere (fig. 4, *b*, *d*) is an elongated, convex, hairy plate; the posterior part (fig. 4, *b*, *d*) is a long bent plate (in side view, fig. 4, *e*) and ends in a blunt point.

♀. Fig. 5. The ovipositor is structurally similar to that of *intestinalis* and *haemorrhoidalis*, the only important difference being that the end of the ninth sternum is markedly expanded. The spermathecae (fig. 5, *b*) are looped, and one is larger than the other.

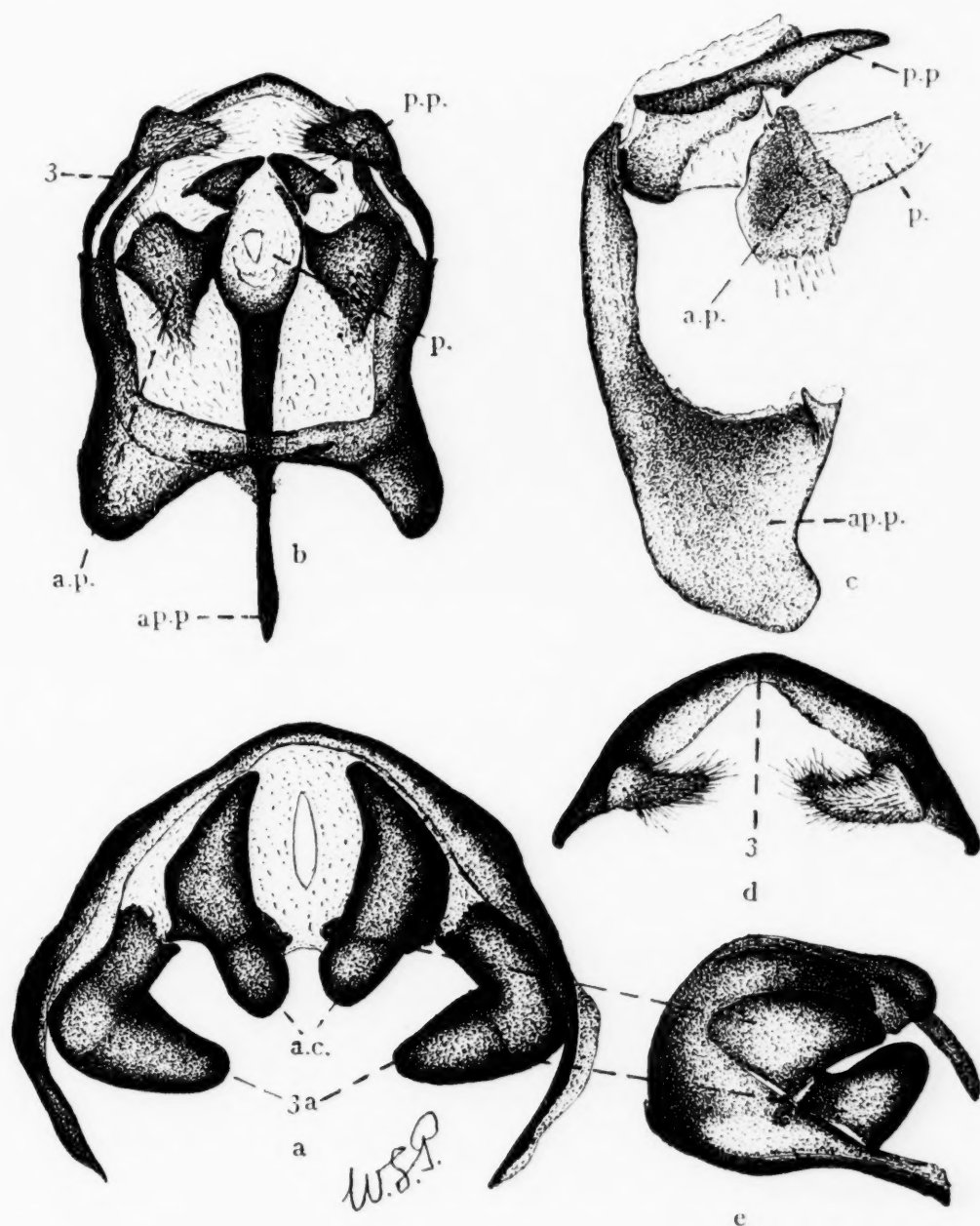


FIG. 6. *a*.—Ventral view of tenth tergum of ♂ *G. pecorum*, showing anal cerci and distal segments of ninth coxites; lettering as in fig. 1, *a*; *b*.—Antero-dorsal view of ninth tergo-sternum, showing phallosome, etc.; lettering as in fig. 1, *b*; *c*.—Phallosome and one paramere in side view; lettering as in fig. 1, *b*; *d*.—The fused proximal segments of ninth coxites, showing finger-like hairy process on each; *e*.—Lateral view of tenth segment, showing anal cercus and distal segment of ninth coxite.

Gasterophilus pecorum F. SALIENT DIAGNOSTIC CHARACTERS OF TERMINALIA.

♂. Fig. 6. The distal segment of the ninth coxite (fig. 6, *a, e*) is a short, broad, rounded plate, bent inwards towards its fellow; the proximal segments (fig. 6, *b, d*) are fused to form an arched plate which is attached to the ninth tergosternum as shown in fig. 6, *b, d*; each has at the side a small hairy plate with the end directed inwards. The anal cerci (fig. 6, *a, e*) are bent elongated plates, their short, rounded, free ends projecting on each side of the anal opening

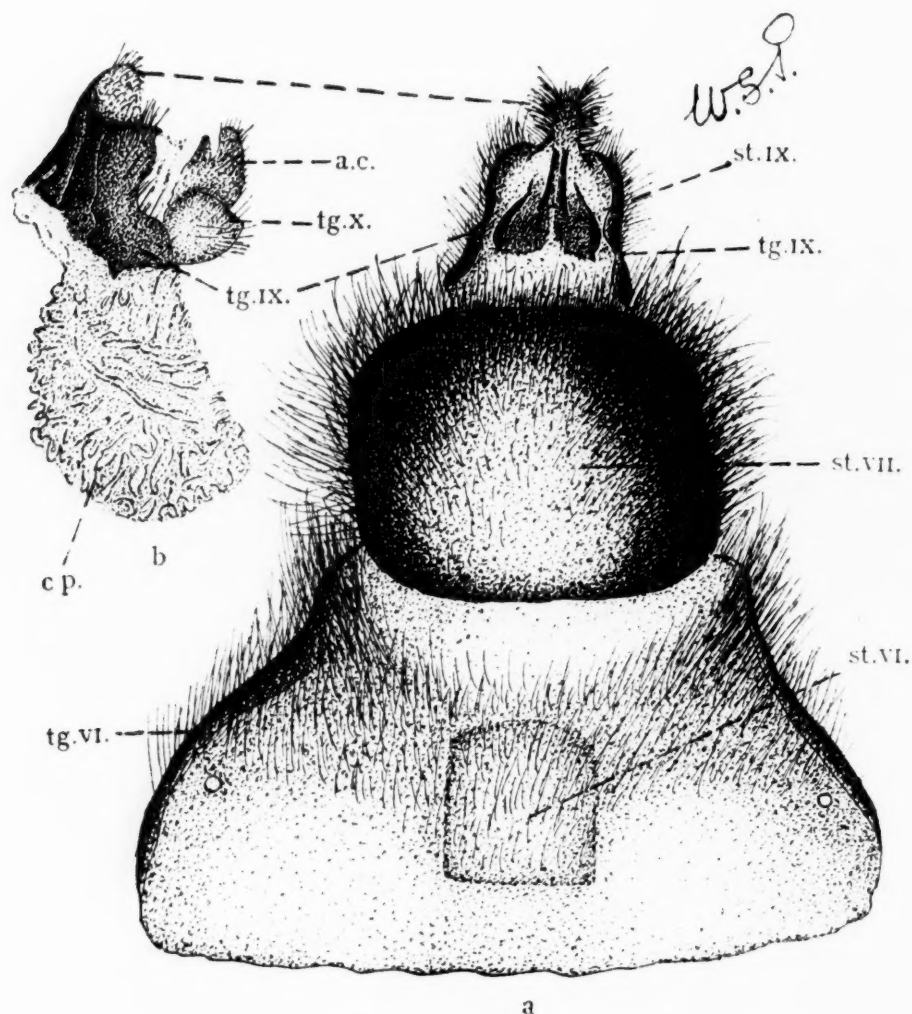


FIG. 7. *a*.—Ventral view of ovipositor of *G. pecorum*; lettering as in fig. 2, *a*; *b*.—Lateral view of end of ovipositor; *c.p.*—Chitinous plate; other lettering as in fig. 2, *d*.

between the distal segments of the ninth coxites. The ninth tergosternum (fig. 6, *b*) is similar to that of the other species, the short, narrow, posterior arms not meeting in the middle line and enclosed by the fused proximal segments of the ninth coxite, the ends of which are hinged in a notch of the ninth tergosternum (fig. 6, *b*). The phallosome (fig. 6, *c*) is small and mainly membranous, the chitinous part consisting of a narrow plate on each side; the apodeme (fig. 6, *c*) is similar to that of the other species. The anterior part of the paramere (fig. 6, *c*) is a small, irregularly shaped, hairy plate; the posterior part (fig. 6, *c*)

in side view is seen to be a narrow elongated plate, lying on each side of the phallosome and ending in a blunt point.

♀. Fig. 7. The ovipositor is relatively short and hairy. Tergum and sternum 7 are fused to form a chitinous tube which is covered by numerous hairs. The distal end of the ovipositor is poorly developed, but is structurally similar to that of the other species; the end of the ninth sternite is not expanded. It is interesting to note that, as has been pointed out by Dinulescu (1929), this species does not lay its eggs on the horse, but most probably on fodder in the stable. Although the eggs have not been found in this situation, and although they have not been seen to be laid on the horse, Dinulescu has noted eggs laid on puparia by females in captivity. The egg is fastened by its posterior extremity to the object by a fibro-gelatinous appendage, which spreads out fan-like on to the surface of the object. It will be noted, then, that, although the end of the ovipositor retains the structural characters of those of the genus, it is poorly developed and is thus not well adapted to lay the egg on a hair, the egg not having any flange for this purpose. Dinulescu has counted from 2,343 to 2,560 eggs in a single female, showing that, as the chance of the larva reaching its host is reduced, owing to the eggs being laid on objects in the neighbourhood of the horse rather than on the animal, a larger number of eggs is necessary.

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SUSCEPTIBILITY OF VARIOUS MAMMALS TO EXPERIMENTAL INFECTION WITH *FASCIOLOPSIS BUSKII* (TREMATODA: FASCIOLIDAE)*

BY

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(Received for publication 28 May, 1937)

It is a well-known fact that pigs as well as human beings are susceptible to infection with *Fasciolopsis buskii* (Lankester) Odhner; but whether other mammals also serve as the definitive hosts of this digenetic trematode seems still obscure. Nakagawa (1922) and Nakagawa and Suzuki (1923) have carried out feeding experiments on pigs in Formosa, and Young (1936) in this country extended these studies to cats, monkeys, guinea-pigs, rabbits and sheep.

In the present experiment, which was started in 1934, 12 species of mammals were subjected to *F. buskii* infestation, viz., 6 pigs, 4 dogs, 1 buffalo, 6 rabbits, 1 monkey, 4 cats, 1 sheep, 1 goat, 1 ox, 5 guinea-pigs, 5 albino rats and 10 mice. They were fed with living encysted metacercariae (Pl. VI, figs. 5 and 6) of *F. buskii* removed from the skin of red water caltrops, *Trapa natans* L. (Pl. VI, fig. 8), collected near Shaohsing, Chekiang Province. The stools of these animals were examined several times prior to infection, and all were found to be free from eggs of *F. buskii*. The food of the animals was not particularly supervised; they were provided with the ample and wholesome diet generally adopted in the animal house of the Institute.

Autopsies were performed at different intervals, and any morbid changes present were carefully noted. The worms found were first studied *in vitro*, then flattened between slides, fixed in 5 per cent. formalin and stained with paracarmine. Measurements (in millimetres) were made on these compressed specimens, unless otherwise stated.

PRESENTATION OF DATA

I. PIGS

Six locally bought pigs, all about three months old, were fed with varying amounts of *F. buskii* metacercariae or cysts (see Table, p. 365). Their daily food consisted of bran (88 per cent.), tankage (6 per cent.) and dried blood (6 per cent.). Stool examinations were started one month after feeding the parasites. It was

*Read before the 3rd annual meeting of the Chinese Society of Pathology and Microbiology, held at Shanghai, April 6th, 1937.

found that the time of appearance of *F. buskii* eggs varied from 78 to 103 days. The animals were autopsied between 176 and 308 days after infection, and practically all the worms recovered were mature specimens located mostly in the duodenum (Pl. VI, fig. 4). Other helminths found together with *F. buskii* in some of the pigs were *Trichuris suis* and *Cysticercus tenuicollis*, both of which were relatively scanty. Body weights of these pigs had not been taken either before infection or at autopsy, but normal development seemed to have taken place.

Average body measurements (length, width and thickness) of 37 living *F. buskii* immediately after removal from the intestine of pig no. 4 were $24.9 \times 11.7 \times 3.7$; their average weight was 17.65 gm.; 19 stained compressed specimens from pig no. 5 averaged 34×12.9 mm.

The gross changes noted at autopsy consisted of minute petechial haemorrhages in the areas where worms were located, and oedema in some other places. Histologically the mucosal glands appeared cystic and in a catarrhal condition. In the acini some mucous exudate was met with, and the columnar epithelial cells were tall and proliferative. A detailed report of these findings will be made in conjunction with Dr. L. S. Kau, the tissue pathologist of the Institute.

II. DOGS

Four street dogs (*Canis familiaris*), averaging 17.4 kgm., were used in this experiment; they were fed with specially made biscuits, the components of which were bran, tankage, bone, white flour and liver oil. More than 154 *F. buskii* cysts were given to each of them (see Table), and autopsies were performed between 152 and 572 days after infection. Only in one dog 4 immature *F. buskii* (Pl. VI, fig. 1) were found in the duodenum—practically without causing any significant pathological changes. Other helminths commonly present in these dogs were *Echinochasmus perfoliatus*, *Dipylidium caninum*, *Spirocerca sanguinolenta*, *Ancylostoma caninum*, *Dirofilaria immitis* and *Clonorchis sinensis*.

Measurements of the four specimens occurring in dog no. 2 were: body size $7.15\text{--}13.3 \times 4.0\text{--}6.1$ (average 11×4.7); pharynx $0.25\text{--}0.47 \times 0.37\text{--}0.56$ (average 0.37×0.50); oral sucker $0.24\text{--}0.42 \times 0.35\text{--}0.52$ (average 0.36×0.44); ventral sucker $1.3\text{--}1.7 \times 1.1\text{--}1.4$ (average 1.5×1.2); Mehlis gland (average) 0.74×0.67 .

The body of these worms was comparatively large; when fresh, they measured 6–13 long, 4–8 wide and 0.2–1.0 thick. They were of an elongated oval shape and pinkish in colour, resembling the mature worms obtained from the pigs. The ventral sucker was bell-shaped and appeared as a powerful organ. Both the oral sucker and the pharynx were subequal in size; the pre-pharynx and oesophagus were exceedingly short.

The testes were very branched, and their ramifications could be seen only faintly even in stained specimens. The cirrus pouch was well developed,

and included the seminal vesicle and ejaculatory duct. The ovary had several branches, but their number was not as large as in mature specimens from pigs. The Mehlis gland was fairly large and was visible to the naked eye. A few vitelline follicles were seen along the body-wall near the ovarian region, but were absent in other parts of the body. No eggs were found in these four individuals.

III. BUFFALO

A young buffalo, about three months old, was obtained from the municipal abattoir at Shanghai; it subsisted on hay. Thousands of *F. buskii* cysts were fed to this animal, but unfortunately it died 20 days after infection, the cause of death being probably septicaemia following acute enteritis. At autopsy 46 immature *F. buskii* were recovered from the small intestine (see Table). Dozens of nematodes were also present.

The immature specimens of *F. buskii* (Pl. VI, fig. 2) were whitish in colour and of rather uniform size. The posterior end of the worm appeared more or less pointed. The ventral sucker was a little larger than the oral one and tended to become bell-shaped as the size of the worm increased. Prepharynx and oesophagus were very short, almost negligible.

The fundamentals of the main genital organs (Pl. VI, fig. 2) were fairly distinct, the two testes especially being represented by band-like structures. Vitelline glands were absent.

Measurements of these specimens were: body size $0.90-1.6 \times 0.40-0.68$ (average 1.3×0.50); pharynx $0.07-0.13 \times 0.09-0.13$ (average 0.11×0.11); oral sucker $0.14-0.17 \times 0.14-0.18$ (average 0.15×0.16); ventral sucker $0.15-0.31 \times 0.15-0.27$ (average 0.23×0.20).

IV. RABBITS

Five mature rabbits (*Lepus cuniculus*), each weighing about 1,750 gm., were bought locally and kept under observation for more than one month. Their food consisted of cooked soy beans, fresh vegetables and carrots. They were given large numbers of *F. buskii* cysts, the exact amount of cysts not being determined. Autopsies were carried out between 52 and 217 days after infection. Except in no. 12, practically no pathological changes were observed in the organs of these animals—not even in the small intestine, where the worms were located. The weights of the animals had remained fairly constant, and no symptoms had been noticed. Three of the rabbits were found to contain *F. buskii* (see Table), the other two proving negative. Among the former, two harboured rather immature *F. buskii* very similar in size and structure to those occurring in the buffalo, while in the third much bigger specimens were found, some of which contained eggs. The results may be summarized as follows:—

Rabbit no. 10. This animal was examined after 110 days of infection. Nineteen immature *F. buskii* were recovered (Pl. VI, fig. 3); they were small,

whitish, and generally pointed at the posterior end. Measurements of these specimens were: body size 0.91–2.0 × 0.42–0.66 (average 1.3 × 0.52); pharynx 0.08–0.12 × 0.10–0.15 (average 0.10 × 0.11); oral sucker 0.13–0.17 × 0.15–0.19 (average 0.15 × 0.17); ventral sucker 0.14–0.38 × 0.15–0.29 (average 0.24 × 0.22); anterior testis (average) 0.07 × 0.12; posterior testis (average) 0.06 × 0.09; Mehlis gland (average) 0.05 × 0.05.

Rabbit no. 13. This animal was autopsied 52 days after infection, when 154 immature specimens were found. All of them exhibited similar development to those in rabbit no. 10, although the former had a shorter period of infestation. Measurements of these individuals were: body size 0.64–1.9 × 0.22–0.82 (average 1.2 × 0.50); pharynx 0.05–0.12 × 0.05–0.13 (average 0.09 × 0.10); oral sucker 0.10–0.17 × 0.13–0.17 (average 0.14 × 0.15); ventral sucker 0.14–0.38 × 0.14–0.30 (average 0.26 × 0.22).

Rabbit no. 12. This animal, 183 days after infection, was very interesting, as some mature *F. buskii* were found. Altogether 32 specimens were present, and practically all of them showed pronounced growth (Pl. VII, figs. 1–8 inclusive). This was indicated by the relative increase in size of the various structures, the measurements being: body size 6.5–22 × 3.0–8.0 (average 16 × 6.0); pharynx 0.25–0.56 × 0.31–0.69 (average 0.37 × 0.44); oral sucker 0.25–0.51 × 0.31–0.71 (average 0.34 × 0.42); ventral sucker 1.2–2.3 × 0.93–2.2 (average 1.6 × 1.3); Mehlis gland (average) 0.65 × 0.51; eggs 0.070–0.125 × 0.051–0.077 (average 0.110 × 0.064). Living specimens were reddish in colour, and moved actively like those found in pigs. Four out of the 32 specimens contained mature eggs of *F. buskii*, varying from a few to hundreds (Pl. VII, fig. 2). The vitellaria and vitelline ducts could be seen in some of the specimens (Pl. VII, fig. 8). The testes, ovary, Mehlis gland, uterus and cirrus pouch of these worms all showed enormous development as compared with those found in the above two rabbits.

Since the morphology of adult *F. buskii* is well known, it would be superfluous to describe the specimens from this rabbit. The general development of the various organs is illustrated by a series of photographs.

Noticeable pathological changes, resembling those found in our pigs, were noted in the duodenum of this rabbit, where most of the worms were accumulated.

V. MONKEY

Only one monkey (*Macacus* sp.) was used in this experiment. It had been bought locally and was fed on cooked rice, carrots and green beans. The cysts of *F. buskii* were introduced into its stomach by means of a long rubber tube, in order to ensure their ingestion. The monkey weighed about 3,580 gm. before infection, and there was not much change afterwards. Autopsy was performed after 563 days, and no *F. buskii* were found. Some *Oesophagostomum* were present.

TABLE

Showing the results of *F. buskii* infestation among pigs, dogs, rabbits and buffalo

Animals	Animal no.	Total cysts fed	Days from first infection to appearance of eggs	Days from first infection to autopsy	<i>F. buskii</i> recovered	Remarks
Pig	4	228	78	176	37	28 matures 9 immatures
	5	205	102	256	45	Matures
	6	525	103	197	5	..
	7	342	78	308	14	..
	9	787	94	213	12	..
	10	203	84	302	0	—
Dog	1	154	—	187	0	—
	2	222	—	201	4	Immatures
	3	320	—	572	0	—
	6	Many	—	152	0	—
Rabbit	10	..	—	110	19	Immatures
	12	..	—	183	32	28 immatures 4 matures
	13	..	—	52	154	Immatures
	14	..	—	217	0	—
	15	..	—	102	0	—
Buffalo	1	..	—	20	46	Immatures

VI. CATS

Four mature cats (*Felis domestica*), averaging 2,753 gm., were bought locally and were fed with large numbers of *Fasciolopsis* cysts. Their diet contained cooked rice, beef and occasionally fresh milk. Autopsies were carried out between 40 and 223 days after infection (average 112 days) and no *F. buskii* were found. Other helminths occurring in these animals were *Clonorchis sinensis*, *Metagonimus yokogawai*, *Prohemistomum appendiculatum*, *Taenia taeniaeformis* and *Physaloptera praeputialis*.

VII. SHEEP

One sheep (*Ovis ammon*), about 8 years old and weighing approximately 67 lb., was employed in this experiment. It lived on ordinary hay. Large numbers of *F. buskii* cysts were fed to it, the animal being killed 147 days afterwards. No *Fasciolopsis* cysts were present.

VIII. GOAT

A goat (*Capra hircus*), about 4 years old and weighing about 38 lb., was fed with numerous *F. buskii* cysts. Its food also was ordinary hay. No *Fasciolopsis* were found 147 days after infection.

IX. OX

An old yellow ox was obtained locally and fed with hay. Numerous *F. buskii* cysts were administered in the ordinary way, and autopsy was made 84 days after infection. *Fasciolopsis* were absent. Other helminths found were *Homalogester paloniae*, *Fischoederius elongatus*, *Schistosoma japonicum* and *Fasciola hepatica*.

X. GUINEA-PIGS

Five mature laboratory-bred guinea-pigs, weighing on an average 542 gm., were given many *F. buskii* cysts. The food consisted of cooked soy beans, cabbage and carrots. They were autopsied between 40 and 122 days (average about 91 days) after infection. Neither *F. buskii* nor other helminths were detected.

XI. RATS

Five mature albino rats (*Mus norvegicus albinus*), weighing on an average about 348 gm., were fed with numerous *F. buskii* cysts. Their diet consisted of a mixture of oats 50, dried blood 1, dried yeast 1, salt 0.05, milk powder 5. Autopsies varied from 50 to 97 days, averaging 78 days. *F. buskii* was absent. The occurrence of *Cysticercus fasciolaris* in the liver of these animals was relatively common.

XII. MICE

Ten mature mice (*Mus musculus*), weighing about 35 gm. each, were fed with *F. buskii* cysts as usual. Their diet comprised a little milk, besides corn-flour. They were autopsied between 2 and 108 days later, averaging 38 days, but no *F. buskii* were found.

GENERAL DISCUSSION

Considering the experimental results outlined above, the question naturally arises of what factor or combination of factors is responsible for the resistance of certain hosts to *F. buskii* infection to which other animals are highly susceptible. Chandler (1932) and Clapham (1933b) have both reviewed the problem of helminth immunity up to that time. It is apparent that animals may exhibit immunity as a result of helminth infestation, but this immunity seems rarely to be absolute. That it can be broken down by artificial means, especially by vitamin deficiency, has been well proved in several cases (Hiraishi, 1928; Clapham, 1933a).

The present study reveals a partial or complete natural immunity against fasciolopsiasis among most of the animals tested. Since the data so acquired are still scanty, it would be unjustified to offer any definite explanations or conclusions. It should be noted, however, that the metacercariae of *F. buskii* can withstand a temperature of 113° F. *in vitro*, whilst the normal temperature of all mammals used does not exceed 107° F. Hence it seems safe to state that the body temperature of the animals used cannot prevent the development of *F. buskii* in them. Other factors, like the influence of age, diet or vitamin deficiency on the host, will have to be appreciated after further experimentation.

For the sake of convenience, the present results may be divided into four groups in accordance with the sensibility of the 12 species of mammals to fasciolopsiasis. Group A is represented by the pig as well as by man; B, by cats, rats, mice and guinea-pigs; C, by the goat, sheep, ox and monkey; D, by dogs, buffalo and rabbits. These will now be discussed *seriatim*.

Inasmuch as in the present experiments relatively few *Fasciolopsis* cysts were fed to pigs, the number of adults obtained was correspondingly small. As a consequence, all the worms recovered were mature, and most of them were found in the duodenum. Symptoms such as diarrhoea, paralysis of the legs, delay of growth among young pigs, as mentioned by Young (1936), have not been noticed by the writer. Since other features of the worms investigated in the present series are fairly comparable to the finding of Young, it is unnecessary to repeat the description here.

There is, however, one point worth mentioning, namely, the so-called physiological strains of *F. buskii* among humans and pigs. The presence of *F. buskii* in the latter has been well confirmed in Formosa (Nakagawa, 1922), Hongkong (Chen, 1935) and India (Chandler, 1928). In all these places the human population as well is infected with the helminth (Maxwell, 1911;

Heanley, 1908). It is interesting to note that 'in the Shaohsing region (Chekiang Province), where many localities show very severe infestation among humans, no pig cases have been found in over 6 years' search' (Barlow, 1925). During the writer's brief stay in the endemic villages of Shaohsing in 1935, he found, however, a pig naturally infected with *F. buskii*. A systematic examination of the pigs in and around Shaohsing is therefore called for, so that quantitative data may be obtained and compared with findings in other regions.

Group B, comprising cats, rats, mice and guinea-pigs, has so far been found to be immune to fasciolopsiasis. Young (1936) also reported that cats and guinea-pigs could not be infected by feeding them with *F. buskii* cysts. The underlying cause of this phenomenon is obscure. Probably these animals have developed an age-immunity, or the good quality of their food may increase their resistance. The intestinal tract of rats and mice is comparatively small, so that it may be unfavourable for the growth of *F. buskii*.

Regarding group C (goat, sheep, ox and monkey), it is rather difficult to say whether any natural immunity against fasciolopsiasis exists, as only one animal has been tested in each case. Young (1936) reported negative results from a sheep artificially fed with *F. buskii* cysts, but he obtained immature worms from a monkey similarly infected. It must be admitted that, if *F. buskii* developed in our monkey, it may have been passed out before autopsy because the animal was kept for too long a period. Sprehn (1932) and Mönnig (1934) included the goat as a final host of *F. buskii*, but in this study the result is so far negative.

The last group of animals, dogs, buffalo and rabbits, are of particular interest. Natural *F. buskii* infection among dogs was reported by Ishii (1929) from Canton, and by Faust (1929) from Siulam, Kwangtung Province. Both Sprehn (1932) and Mönnig (1934) in their text-books have included dogs as final hosts of *F. buskii*. Nakagawa and Suzuki (1923), Young (1936), and the present writer, however, found only immature *F. buskii* in several experimentally infected dogs. Vogel (1936) fed 100 *F. buskii* cysts to each of two dogs, as well as 40 cysts to himself, with the results that none developed in the dogs, but mature worms were present in himself. In view of these experiments carried out in different places, it seems improbable that dogs may serve as carriers of fasciolopsiasis in any endemic region. The eggs of *Echinochasmus perfoliatus* commonly found in dogs closely resemble those of *F. buskii*, although they are usually smaller. This may account for the reported occurrence of *F. buskii* in dogs when only stool examinations were made.

Our experience with the buffalo is far from being conclusive, as the single animal used was very young and, moreover, died too early. It is, however, significant to note that its worms, though still in immature stages, exhibited signs of growth. Whether or not they will develop further, remains to be elucidated.

Among the five rabbits fed with *F. buskii* cysts, at least one proved definitely infected, as evidenced by the presence of eggs in the worms and the morbid

changes produced in the host. In this respect the writer's results differ from those of Young (1936), who found only immature *F. buskii* in three rabbits examined 185 days after infection. It would be premature to ascribe this discrepancy to individual variations or dietary factors. Further studies are called for.

SUMMARY

Twelve species of mammals, represented by 6 pigs, 4 dogs, 1 buffalo, 6 rabbits, 1 monkey, 4 cats, 1 sheep, 1 goat, 1 ox, 5 guinea-pigs, 5 albino rats and 10 mice, were fed with *F. buskii* cysts and autopsied at different intervals. Practically all helminths develop to maturity in pigs when these are lightly infected. As far as was ascertained, the worms remain immature in dogs. In rabbits, both immature and mature stages of *F. buskii* were met with, and there is a possibility that this host may prove a suitable experimental animal for *Fasciolopsis* work. Immature worms were noted in the young buffalo, but there is not sufficient evidence to say whether they might develop further. The remaining animals (monkey, cats, sheep, goat, ox, guinea-pigs, rats and mice) appear at present resistant to *Fasciolopsis* infection.

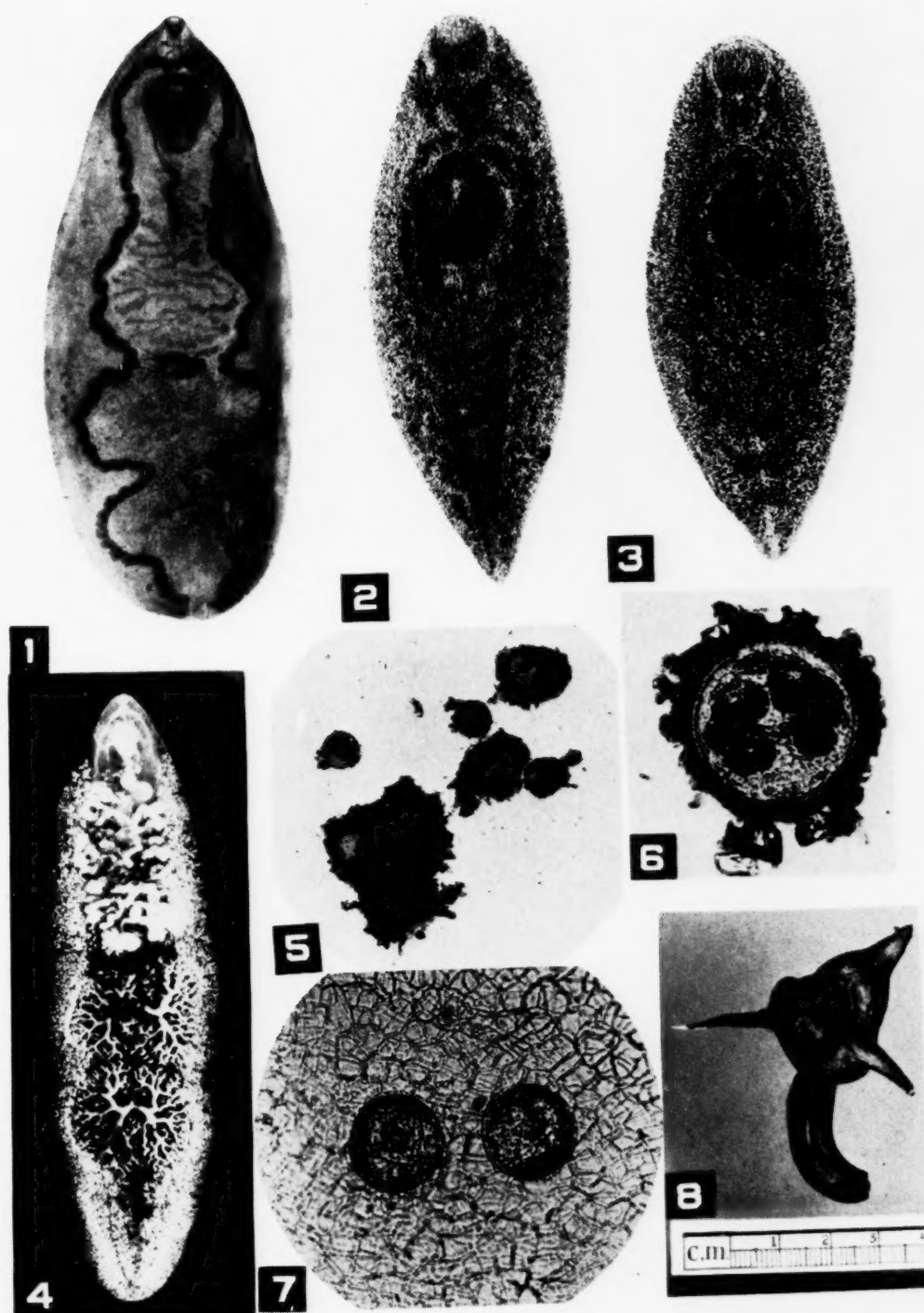
ACKNOWLEDGEMENTS. The writer wishes to record his appreciation to Dr. H. G. Earle, Director of the Institute, and to Dr. R. C. Robertson, Head of the Division of Pathological Sciences, for their interest in this investigation. Thanks are also due to Dr. J. Edgar and Dr. L. McWhirter, of Keylock and Pratt Infirmary, Shanghai, for the supply and maintenance of four animals (buffalo, ox, goat and sheep) during this investigation.

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EXPLANATION OF PLATE VI

- Fig. 1. Immature *Fasciolopsis buskii* experimentally obtained from a dog. ($\times 10.5$.)
- Fig. 2. Immature *F. buskii* experimentally obtained from a buffalo. ($\times 72$.)
- Fig. 3. Immature *F. buskii* experimentally obtained from a rabbit. ($\times 72$.)
- Fig. 4. Mature *F. buskii* experimentally obtained from a pig. ($\times 2.75$.)
- Fig. 5. Living metacercariae of *F. buskii* removed from the skin of red water caltrops. ($\times 84$.)
- Fig. 6. A living metacercaria of *F. buskii*. ($\times 414$.)
- Fig. 7. Two stained metacercariae of *F. buskii*. Note the vegetative structure in the background. ($\times 228$.)
- Fig. 8. A red water caltrop, *Trapa natans* L.

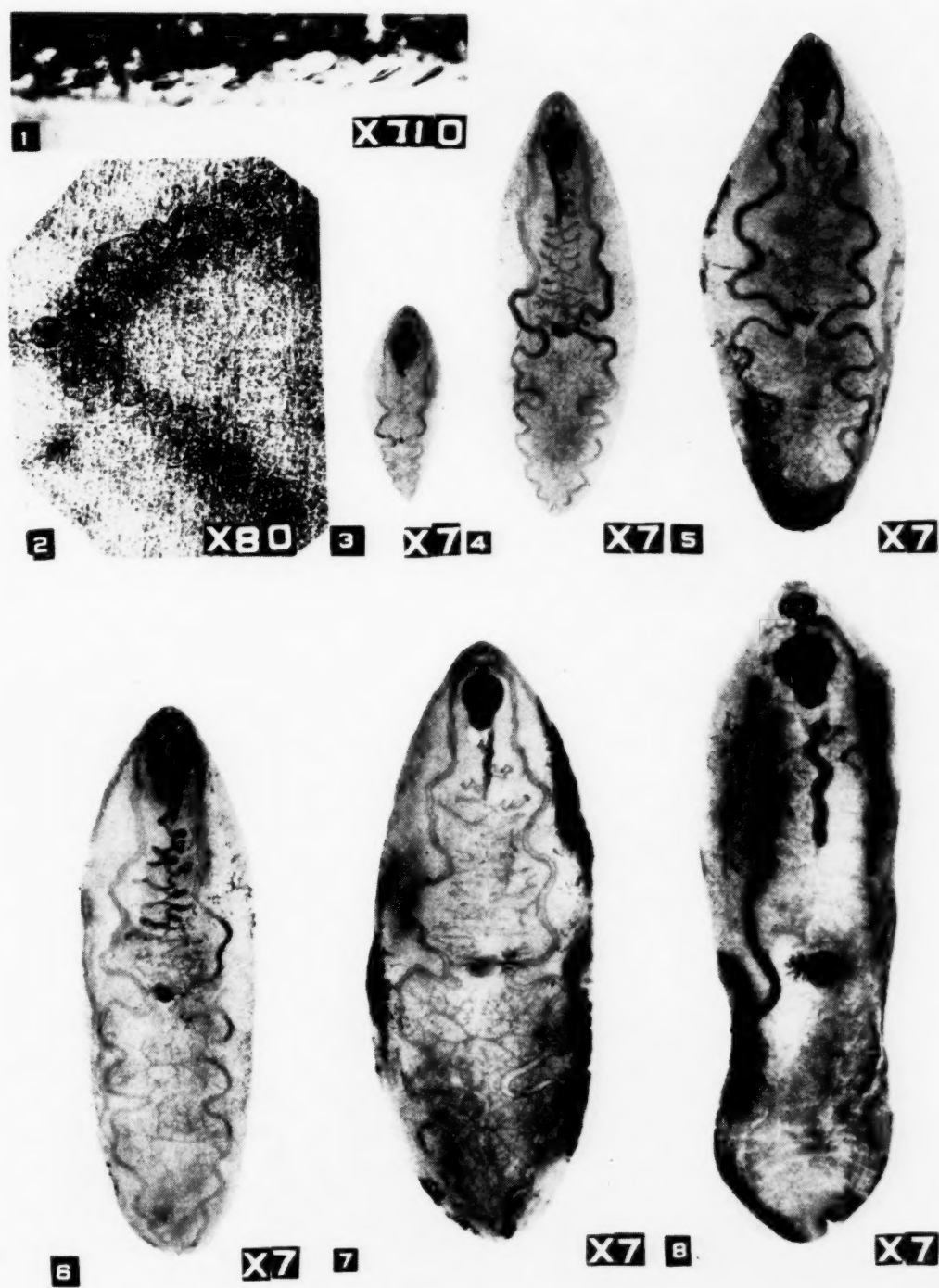


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EXPLANATION OF PLATE VII

- Fig. 1. Cuticular spines along the body margin of *F. buskii* experimentally obtained from a rabbit. ($\times 710$.)
- Fig. 2. Mature *F. buskii* eggs in utero. ($\times 80$.)
- Figs. 3-5. Immature *F. buskii* experimentally obtained from a rabbit. ($\times 7$.)
- Figs. 6-8. Mature *F. buskii* experimentally obtained from a rabbit. Note the vitelline follicles along the body margin of the worm in fig. 8. ($\times 7$.)



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VACCINATION IN YELLOW FEVER WITH NON-INFECTIVE VIRUS

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(Received for publication 30 July, 1937)

Immunization by means of lifeless vaccines has been accomplished less successfully in the virus diseases, *sensu restrictu*, than in the case of some of the ordinary bacterial infections. The technical problems presented by the virus diseases are difficult, but yellow fever presents favourable opportunities in some respects for the study of immunization by the use of killed virus.

MATERIAL AND METHODS

Preparation of the Vaccine. The French neurotropic strain of yellow fever has been used exclusively in these observations. It has been propagated by serial inoculation in ordinary mixed breeds of white mice, using animals at varying intervals from the 271st to the 350th passage. A suspension was prepared in which the total volume contained 20 per cent. by weight of the finely triturated infective brain. This was made up in physiological saline solution containing 1 per cent. of phenol; the vaccine was aged for 6 days at 37° C. and then stored at a temperature of about 4° C. This plan follows, except in detail, the technique adopted by Fermi (1908) and by Semple (1911) for the preparation of a phenolized vaccine for rabies. At the expiration of six days in the incubator, each lot of vaccine was tested for infectivity by the intracerebral injection of 12 to 24 mice; these numbers do not include those animals which died from the effects of the phenol. About 1/60 c.cm. of the sediment of brain tissue was used for each mouse, as it would be in the particles of tissue that virus might occasionally survive. No mice developed infections under these conditions. The stock of vaccine was then diluted with an equal volume of physiological saline solution before use for the immunization of animals. This technique was modified as described later in some experiments involving the use of cysteine hydrochloride.

If the methods employed for killing the virus are on the border-line of efficiency, then traces of living virus in some samples of the resulting vaccine may easily escape detection. Errors in sampling may occur even when large amounts of vaccine are tested for infectivity; instructive experiences of this nature have been recorded in some careful observations by Gordon and Hughes (1936). We have made some observations on the effect of ageing without the

use of phenol, and also on the length of time ordinarily required for 1 per cent. phenol to render the virus non-infective. A 20 per cent. suspension of infective mouse brain in 0.85 per cent. salt solution was incubated at 37° C. Tests made by intracerebral injection at the end of 4 days resulted in the death of half of the mice which were inoculated, but at the end of 6 days the suspension was innocuous. A 20 per cent. suspension of infective brain in 1 per cent. phenol was allowed to stand for 20 minutes at room-temperature; injections were then made intracerebrally into 6 mice, and all died of encephalitis, though their death was delayed from 4 to 5 days as compared with the corresponding controls. On two occasions the 1 per cent. phenolized vaccine before dilution with saline was tested intracerebrally on mice after 24 hours incubation at 37° C., and all the animals remained well. There remains the usual question whether a virus which is no longer infective is necessarily dead; the conservative designation of this condition as complete inactivation might indicate that the virus had also lost its antigenic properties.

Immunization of Animals. White mice of mixed breed were injected subcutaneously with the 1/2 per cent. phenolized vaccine in relatively large doses. Tests for active immunity by the intraperitoneal route were made with a 10 per cent. suspension of neurotropic yellow fever virus, in physiological saline containing 10 per cent. of normal serum. This was centrifugalized, and 0.2 c.cm. of the supernatant fluid was injected with simultaneous traumatization of the brain by the intracerebral injection of starch paste (2 per cent. in water). One group of vaccinated mice was tested by the injection of virus into the brain.

Two rabbits were injected subcutaneously with vaccine over moderately long periods of time. Subsequently their serum was tested for its ability to confer passive protection in mice. These neutralization tests were carried out by the intraperitoneal route. A 10 per cent. suspension of infective mouse brain in serum-saline was centrifugalized at moderately high speed and only the supernatant fluid was used. A somewhat similar technique has been described by Snijders, Postmus, and Schüffner (1934). A mixture was made consisting of equal parts of the infective supernatant fluid and of the serum to be tested. The brain was injected with starch paste, and 0.5 c.cm. of the mixture of serum and virus were injected intraperitoneally. In some instances, titrations of the sera were made at dilutions of 1 to 10, 1 to 50, 1 to 100 and 1 to 250, these figures representing the final dilution of the serum after admixture with the virus. These protection tests were carried out with the Swiss strain of white mice. In all these experiments, the mice which received living virus were kept under observation for a period of from 3 to 4 weeks. The control animals used in each experiment are not always mentioned specifically, except in those instances where some of the controls survived.

One may consider briefly the possibility that the infective mouse brains used for the preparation of the vaccine might also contain some specific protective substances against yellow fever and thus confer passive immunity. Hoskins

(1935) found no evidence of the presence of antibodies in the brains of mice dying of neurotropic yellow fever. In the following experiments, the animals were not tested for immunity until at least 3 weeks after their last injection of vaccine, except where occasional tests were made with sera of rabbits during the course of their immunization.

RESULTS OBTAINED IN ANIMALS

Mice. For a preliminary test, 8 mice, of an average weight of 20 gm., were given subcutaneously 1 c.cm. of vaccine, containing 0.5 per cent. phenol; several of them promptly showed symptoms of poisoning, and one died overnight. The dosage was then reduced to 0.5 c.cm. injected at weekly intervals until a total of 2 c.cm. of vaccine had been given. An intraperitoneal immunity test resulted in the death of 3 of these 7 mice, whereas 5 of 6 controls died of encephalitis. A second group of mice was injected subcutaneously at weekly intervals until each animal had received a total of 4 c.cm. of vaccine. The intraperitoneal immunity test resulted in the death of 3 of 10 animals, whereas 6 of 7 controls died. In a third experiment, 18 mice received 4 c.cm. of vaccine divided as before into 0.5 c.cm. doses at weekly intervals. The immunity test was made by the intracerebral injection of about 1/60 c.cm. of the supernatant fluid of a 1 to 10,000 dilution of infective mouse brain. Six of these 18 mice remained well, whereas all of 18 controls injected with this dilution of virus died within 6 days. At a dilution of 1 to 100,000 the virus killed only 3 of 6 normal mice, and the same result was obtained with a 1 to 1,000,000 dilution of the virus. In the interpretation of these data, it is well to remember that mice are immunized only with some irregularity and inconstancy, even when injected with rather liberal amounts of living virus of yellow fever.

Rabbits. A sample of blood was taken from a young rabbit (no. 1) weighing 840 gm. Vaccine in 20 c.cm. amounts was injected subcutaneously at weekly intervals until 120 c.cm. had been given. The injections were well tolerated, the weight of the rabbit increasing to 1,660 gm. The serum taken after vaccination failed to protect mice; the onset of symptoms and death were merely delayed for 1 to 2 days, as compared with the control mice receiving the serum which was taken from this rabbit before any injections were given. After a rest-period of one month, this rabbit was given an additional 100 c.cm. of vaccine subcutaneously in dosages of 20 c.cm. injected twice weekly. Three weeks later the serum protected 5 of 6 mice, whereas 5 of 6 controls died. Another specimen of serum taken 6 weeks after the last injection of vaccine protected only 3 of 6 mice. It appeared that any protective action which this rabbit had developed was rather transitory. However, a third test was made 16 weeks after the final injection of vaccine, and 5 of 6 mice remained well; six months after vaccination the serum of this rabbit, now weighing 2,500 gm., protected 5 of 6 mice, whereas 5 of the 6 controls died of encephalitis.

Vaccine Prepared with Cysteine. The following experiment is based on some important observations of Dr. Mudd concerning bacterial antigens. The purified surface antigen recovered from disintegrated cultures of *Streptococcus haemolyticus* oxidizes with extreme ease; the reaction is reversible, and the activity of the antigen may be restored by reducing agents such as cysteine (Mudd, with Czarnetzky, Pettit and Lackman, 1937). Dr. Mudd and his collaborators at the University of Pennsylvania have very courteously placed their results at our disposal.

An additional lot of vaccine was prepared according to the routine already described, except that cysteine hydrochloride was added in the proportion of 0.1 gm. to 5 gm. of infective mouse brain. The incubation at 37° C. was omitted, and the ageing in 1 per cent. phenol was carried out in the cold room at a temperature of about 4° C. Two similar suspensions of infective brain were prepared; to one of them phenol only was added, and to the other cysteine hydrochloride only, in order to test separately the activity of these agents in destroying the virus. After storage in the cold room, tests for infectivity of these three preparations were made at various intervals by intracerebral inoculations in mice. After an interval of 6 days, the suspension in phenol (1 per cent.) was injected into 5 mice, and all died on the 8th day of encephalitis. Nine mice were injected with material exposed to the action of cysteine hydrochloride for 6 days, and all of these animals remained well. In the presence of both cysteine hydrochloride and phenol, the virus was inert at the end of 3 days, a result that is indicative of the greater activity of phenol in acid solution; after storage for 3 weeks in the cold room, 0.5 c.cm. of the sedimented brain tissue was injected intracerebrally, this amount being divided about equally among 24 mice. All of these animals remained well. The stock of vaccine was then diluted with an equal volume of physiological salt solution. Rabbit no. 2 (weight 2,400 gm.) was given subcutaneous doses of 20 c.cm. of vaccine twice weekly until a total of 140 c.cm. had been injected. One protection test was made during the course of immunization. Serum collected before vaccination was used for a control; the specimen to be tested was taken after 100 c.cm. of vaccine had been given. Five of 6 mice were protected and all of the controls died; at a dilution of 1 to 10 the serum gave no protection. Three weeks after the last injection of vaccine another test showed complete protection at a dilution of 1 to 10 and partial at 1 to 50, but at higher dilutions the number of mice which survived was not significant. At an interval of two months after the last immunizing injection, the serum of this rabbit protected 5 of 6 mice; at a dilution of 1 to 10, 4 of 6 mice survived; but at a dilution of 1 to 50 only 1 mouse lived. Normal horse serum was used for the controls, and 5 of these 6 mice died of encephalitis. Rabbits when given a single injection of a minute amount of living neurotropic virus of yellow fever develop a protective serum promptly and with regularity.

Technical Difficulties of Immunization with Killed Viruses. Various

investigators have emphasized the difficulty of obtaining viruses in adequate concentration for immunization with killed vaccines. There is as yet no clear indication that massive amounts of killed virus are necessary to induce protection, though it is often necessary to use gross amounts of infective tissue in which the quantity of virus is by no means excessive. As a source of material for lifeless vaccine, infective tissues are at a disadvantage when contrasted with pure cultures of bacteria. Thus, a standard vaccine for typhoid and paratyphoid fever may contain a total of 2,500 million bacteria per c.cm. By way of comparison, mice dying of neurotropic yellow fever show an exceptionally high concentration of virus in the brain; under favourable circumstances, one brain may contain an estimated amount of 15,000,000 minimal infective doses of virus. If we compare a minimal infective dose with a single bacterial organism then, at this titre, more than 100 mouse brains (166) would correspond to 1 c.cm. of typhoid vaccine. Also, an additional correction might be appropriate because of the discrepancy in size between the particles of virus as compared with ordinary bacteria. Apart from the problems of obtaining viruses in adequate concentration, there remains the necessity of preserving the antigenic activity of lifeless vaccines. Encouragement in this direction is afforded by the successful results of Mudd and his collaborators.

Evidence has gradually accumulated in several of the virus diseases that immunization, though sometimes of a very low grade, can be obtained by the use of virus which has been rendered non-infective. Findlay and Mackenzie (1936), using a formalinized yellow fever virus, have reported the apparent immunization of 1 of 4 hedgehogs; in one group of 50 mice, 33 were protected. In human medicine, rabies is the outstanding example of successful immunization by means of an 'inactive' virus, used extensively in the form of the phenolized vaccine of Semple (1911). There are several factors which tend to complicate the interpretation of the data concerning this method. Webster (1936) has noted the failure of some samples of commercial phenolized vaccine to produce humoral immunity when tested carefully under experimental conditions. Furthermore, there are two sources by which living virus may enter into the process of immunization. Exceptionally, commercial preparations of phenolized rabies vaccine, though supposedly non-infective, have been shown to contain traces of living virus in some of the grosser particles of brain tissue. The other source of error is even more obvious. Successful prophylaxis of rabies by phenolized vaccine is frequently cited as evidence of immunization by killed virus. However, it is evident that any patient who is protected by this treatment receives also an injection of living street virus, introduced by the bite of the rabid animal. The literature contains experimental data concerning the protective power exhibited by the sera of persons after vaccination with killed rabies virus, but with no indication whether such persons were patients who had been bitten by rabid animals or were experimental subjects entirely free from any exposure to living virus. Unfortunately, the lack of such information mars the

value of otherwise valuable data. Nevertheless, there is a considerable amount of satisfactory evidence that killed virus of rabies can, under suitable circumstances, induce an active immunity. This is of special interest because the fixed virus of rabies does not reach any remarkable concentration in the brain of an infected rabbit. It is difficult to obtain sufficient data for the accurate estimation of the number of minimal infective doses originally present in the infective tissue used for the preparation of the Semple type of vaccine. In a full course of treatment the total quantity of virus, if this can be judged by the number of minimal infective doses, does not approach the order of magnitude of the concentration of micro-organisms in a bacterial vaccine.

SUMMARY

Mice were injected with liberal amounts of neurotropic virus of yellow fever which had been rendered non-infective. Immunity tests resulted in the survival of the vaccinated mice in significant numbers. The sera of two rabbits after many injections with non-infective yellow fever vaccine afforded slight but definite protection to mice as determined by neutralization tests.

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FUNCTIONS OF THE OESOPHAGEAL DIVERTICULA OF *STEGOMYIA AEGYPTI* AND *ANOPHELES TARSIMACULATUS*

BY

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(*Received for publication 3 August, 1937*)

The oesophageal diverticula of the mosquito consist of three sac-like appendages opening into the oesophagus, two dorso-laterally and one ventrally. In the newly hatched insect they are collapsed and retracted, but in the full-grown adult they are filled with air. In structure they consist of a delicate internal layer, in which, with careful focussing, cells may be seen; externally there is a thin muscular coat. Little or nothing is known of the part which they play in the physiology of digestion, though the solution of many of the important problems dealing with the transmission of parasitic blood diseases by insects depends upon a knowledge of the processes and changes taking place in the alimentary tract of the transmitting host during ingestion and digestion. The difference of opinion on the primary matter as to whether blood enters, and is present in, the oesophageal diverticula is still considerable. Nuttall and Shipley (1903) quote Grassi as saying that he found the oesophageal diverticula to contain air mixed with a little colourless fluid, or blood if the insect was examined immediately after sucking blood; they quote also Grandpré and Charnoy, who found them with blood at times if the stomach was replete with blood. By feeding *Culex pipiens* and *Anopheles* on blood serum and sugar coloured with dyes, Nuttall and Shipley (1903) found that these substances when ingested enter the diverticula, which are, therefore, food reservoirs. They concluded that 'the greater part, or all the material ingested, may find its way into the sacs [diverticula], and is thence gradually supplied to the digestive canal proper.' Patton and Cragg (1913) maintain that in the mosquito they are true 'food reservoirs,' and that in insects killed during the act of feeding they are always to be found full of blood, though, subsequently, depending on the rate of digestion and temperature, blood is almost entirely confined to the midgut. Cragg (1920) showed that in the case of *Tabanus* they are not food reservoirs, though during digestion some of the contents of the mid-intestines may be forced into them and again be returned by them to the former. In *Tabanus*—a blood-sucker—they were never found distended with blood. Roy (1927), working with *Anopheles* in India, showed that blood can easily be detected in them after a full meal, and may be found even 4 hours later. On the other hand, Wright (1924) in his work on British mosquitoes obtained discordant results, and states that he has never encountered any mosquito which had the least trace of blood in the oesophageal diverticula. Macgregor (1927) also says

that, though they are in fact food reservoirs, he has never found them to contain blood. By feeding mosquitoes first with fruit and then with blood, or vice versa, he found that the blood enters the stomach, while the fruit-sap enters the diverticula. They function, therefore, solely as reservoirs of fruit-sap and water, and not of blood. Macgregor again (1930), by a novel method of artificially feeding mosquitoes, found that the normal destination of blood in the act of 'biting' through the skin is the stomach, whereas the normal destination of water, fruit-sap and other sugar-containing fluids is the diverticula. Boissezon (1930) is of the opinion that, in the case of *Culex pipiens*, if any blood enters the oesophageal diverticula it is the excess from an overloaded stomach suppressing the sphincter action and allowing some blood cells to enter them. Normally blood cells (7.5μ) are unable to pass through the narrow mouth (3μ) of the diverticula, though smaller particles may. Coloured dyes, when ingested, enter them.

In view of these discordant results, the following investigation was undertaken in Trinidad. Adult insects reared from pupae were caught in test-tubes, and after about 12 hours were allowed to feed on the human forearm at room-temperature (ca. 27.5°), until engorged and the proboscis withdrawn. For special purposes some were interrupted during the feed and fixed immediately. The live insect was at one and the same time killed and fixed, at various intervals of time after the feed, by pouring into the test-tube warm Bless fluid, as recommended by Patton. Serial paraffin sections were cut in the ordinary way and haemalum and oesin were used as stains. It was assumed that the fixation of the whole insect would give a truer picture of the relation of parts than that obtained by tearing and dissection of tissues.

The work here recorded on the functions of the oesophageal diverticula shows that there is a difference between the *Stegomyia* and *Anopheles*. Immediately after a full meal the diverticula of *Stegomyia* become distended and filled with blood cells and are pressed closely against surrounding structures (Pl. VIII, figs. 1, 2, 3, 4). Their walls are thinned and their morphological structure is unrecognizable, but the contained blood cells are discrete and separate, though closely packed together. It is not merely the excess of the ingested blood from an over-distended stomach which passes on into the diverticula. The contained blood does not fill them entirely, for loculi or pockets of air can be seen (Pl. VIII, figs. 2, 3, 4). Within half an hour after a full meal they empty themselves of their blood contents. In the *Stegomyia*, therefore, blood does not enter and remain in these appendages, which are, in fact, true food reservoirs. On the other hand, the oesophageal diverticula of the *Anopheles* are never distended, or appreciably filled, with blood immediately after a full meal and even with an engorged and distended stomach. In some instances, however, a few blood cells may be seen in them, but they are so scanty as to lead to the suspicion that they are regurgitated from the stomach. This opinion is supported by the fact that, while they are absent in the diverticula immediately after a full meal, they

may be present 15 minutes (Pl. VIII, fig. 6) or one hour after a meal (Pl. VIII, fig. 7). That regurgitation of blood does take place—maybe in the act of dying from the chemical fixative—is further proved by the fact that blood cells may be found in the oesophagus and cardia 5 hours after a meal (Pl. VIII, fig. 8), though absent at an earlier period. Immediately after the meal, the diverticula with their loculi may be seen extending along the length of the cardia and stomach, but free of blood and containing, in the opinion of Macgregor, not CO₂, but air (Pl. VIII, fig. 5). With careful focussing, the cells in their walls can be recognized.

This functional difference in the oesophageal diverticula of the *Stegomyia* and *Anopheles* serves to emphasize the fundamental differences in the physiology of digestion in these two insects, corresponding, perhaps, with the marked and selective differences in their ability for parasitizing blood parasites.

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EXPLANATION OF PLATE VIII

Stegomyia

(Immediately after a full meal)

- Fig. 1. Diverticula filled with blood cells. ($\times 188$.)
 Fig. 2. The same, but with loculi. ($\times 47$.)
 Fig. 3. The same as fig. 2. ($\times 188$.)
 Fig. 4. Diverticula, secreting cells of cardia and stomach. ($\times 188$.)

Anopheles

- Fig. 5. Diverticula with loculi, but free of blood. ($\times 47$.)
 Fig. 6. Blood in diverticula 15 minutes after a meal. ($\times 188$.)
 Fig. 7. The same, 1 hour after a meal. ($\times 188$.)
 Fig. 8. Blood in oesophagus and cardia 5 hours after a meal. ($\times 188$.)

EXPLANATION OF LETTERING

- | | |
|---|-------------------------------------|
| a = Cardia with blood cells. | e = Salivary gland. |
| b = Oesophageal diverticula with blood cells. | f = Oesophagus. |
| c = Oesophageal valve. | g = Muscle tissue. |
| d = Loculi in oesophageal diverticula. | h = Junction of cardia and stomach. |



Fig. 1



Fig. 2



Fig. 3



Fig. 4



Fig. 5



Fig. 6



Fig. 7

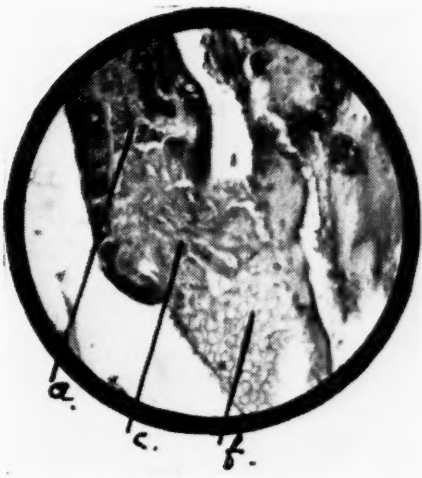


Fig. 8

NOTES ON VARIATION IN *ANOPHELES RIVULORUM* LEESON IN EAST AFRICA, WITH DESCRIPTION OF A NEW VARIETY

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As stated by Evans and Garnham (1936), a considerable amount of variation exists among the series of *Anopheles rivulorum* collected and reared in two different localities in Kenya. More recently, one of us (H.S.L.) has studied material including a number of isolations which he and Mr. J. D. Gillett have obtained in various parts of Uganda and Tanganyika and in the islands of Zanzibar and Pemba, and further light has been thrown on the question of differentiation in this species. Through the kindness of Dr. and Mrs. Bagster Wilson one of us has been able to study further material collected and reared in the neighbourhood of Moshi, Tanganyika Territory.

It is found that many of the characters by which the series appear to be distinguished are unstable, and that a considerable number of intergrading local forms exist. On the other hand, two fairly distinct groups of these series can be recognized by a few characters which are correlated, and, as typical examples of either form are characteristic in some respects, the group which differs from the original series is here described as a distinct variety. It is named in honour of Dr. P. C. C. Garnham, who discovered it at Kisumu and has done a great deal to elucidate its life-history and relation to disease.

A. rivulorum Leeson type form

ADULT. *Wings.* Costa without any basal pale interruption. First vein characteristically with a dark spot separating the pale sector spot from the accessory sector (fig., A); occasionally it may be minute or bridged across by pale scales. (This dark spot will be referred to hereafter as the 'preaccessory dark spot' (p.a.).) Upper branch of fifth vein entirely dark between the cross-vein and apical pale spot. (A pale area in this position will be referred to hereafter as the 'submedian pale spot' or 'area'.)

PUPA. *Accessory paddle hair* with 3 or more branches. Spine A on VII about half or rather more than half length of segment.

LARVA. Anterior dark band of fronto-clypeus not deeper than in the type form of *funestus*. Paired accessory tergal plates present, at least on segments V–VII, and sometimes also on one or all of the preceding segments.

VARIATION. Material collected by one of us (H.S.L.) at Abbia, Uganda, differed from the original Southern Rhodesian series in having the paired accessory platelets sometimes present on all or almost all of the segments (not on I–III in any of the Rhodesian specimens).

BREEDING PLACES. Larvae of *A. rivulorum* were collected from a stream at Ajumani, the River Aswa at Abbia and the River Ania at Arua, all in Uganda. Larvae were among grass at the edges of the streams, where there was no *Pistia* or other floating plant. At Ajumani and Abbia the larvae were associated with *A. funestus*, *A. lesoni* and *A. coustani*. At Arua the only species taken with *A. rivulorum* was *A. funestus*.

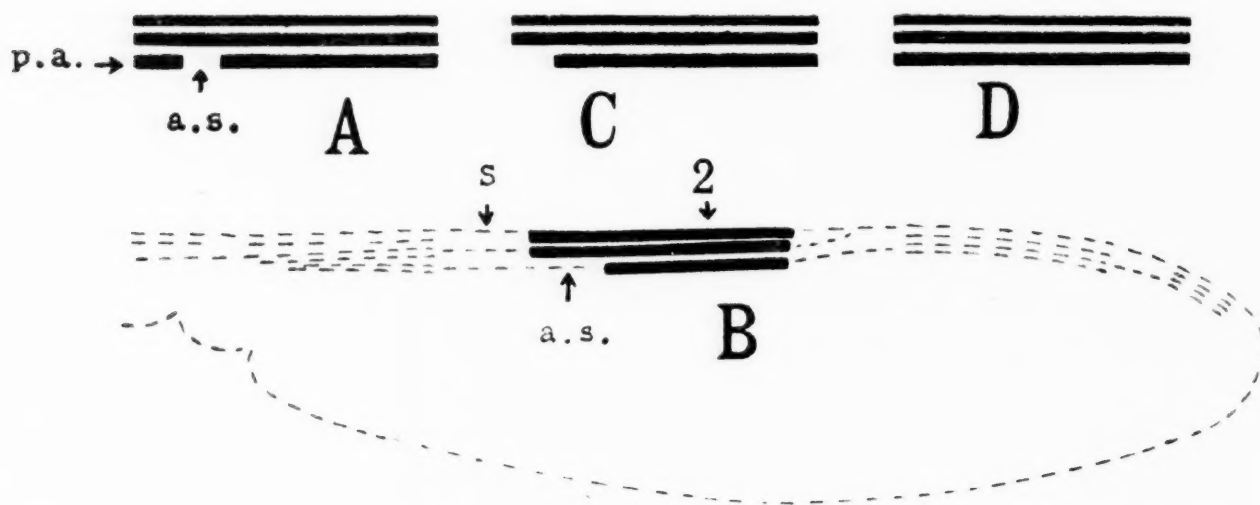


FIG. Showing conditions of the second main dark area on the first vein in the *rivulorum* series of *Anopheles*. A.—*A. rivulorum* Leeson; B and C.—*A. rivulorum* var. *garnhamellus* var. nov.; D.—Very rare.

A. rivulorum var. *garnhamellus* var. nov.

This variety differs from the type form as follows:

ADULT. *Wings*. Costa with at least one basal pale interruption present in a proportion of the specimens of each series. First vein almost always without a dark preaccessory sector spot; pale accessory sector spot usually present and continuous with the sector spot (see fig., B, and next paragraph). Upper branch of fifth vein with submedian pale spot of varying size in about half or more than half the specimens of the series.

It should be mentioned here that in this series of *Anopheles* there are four main conditions of the second dark area on the first vein. These are illustrated

in the fig. In A there is a well-developed pale accessory sector spot (a.s.), which is separated from the sector spot (s.) by a dark preaccessory sector spot (p.a.) (this may be minute or bridged across by pale scales). In B the accessory sector is present and continuous with the sector spot (s.). In C there is no true accessory sector, but a short extension of the sector spot. In D there is no accessory sector and no extension of the sector spot, the dark area being exactly like that on the costa. In the present variety the conditions A and D are very rarely present; the condition B is the commonest, but C and states intermediate between C and B are found.

PUPA. *Accessory paddle hair* tending to have fewer branches. Spine A sometimes much shorter than in type form, but varying in different forms.

LARVA. Anterior dark band of fronto-clypeus variable in width, but commonly so wide as to occupy most of the anterior half. Paired accessory tergal plates absent in a varying proportion of the larvae of the different series, and rarely present on more than 2 or 3 segments. Among 14 Kisumu isolated specimens in which the character was clearly seen, the paired platelets were absent from all but one specimen.

BREEDING PLACES. At Kisumu the variety breeds exclusively in association with *Pistia*; the statement made by Evans and Garnham (1936) has been confirmed by further observations by the latter author. In the Digo district (Evans and Garnham, 1936) the variety is not associated with *Pistia*, but it may be mentioned that observations by one of us (A.M.E.) on nine swamps at 'Msambweni tend to show that the plant does not occur in this district.

In Uganda larvae of *A. rivulorum* var. *garnhamellus* were taken from the River Nile at Atura, Laropi, Rhino Camp and Pakwach, from the River Aswa at Abbia, and from a swamp-pool near Arua. In Tanganyika larvae were found in pools in swamps at Mwanza and Kilosa; and at Jangwani and Msimbazi near Dar-es-Salaam and at Arush-chini. In Zanzibar larvae were collected from two swamps, one of which was caused by a large spring. In all these places, except the swamps at Jangwani, *Pistia* was growing, and at Mwanza rice was growing through the *Pistia*.

The water of the Nile was clear and moved slowly through the *Pistia*, and the temperature varied between 26° C. and 28° C. The water in the swamps was usually clear, with no perceptible movement, and the temperatures were between 23° C. and 27° C., though the rice-swamp at Mwanza had a temperature of 28° C. at the time when the larvae were collected. The salinity of the clear swamp-waters was 10 parts of chlorine per 100,000 parts of water. Two swamps at Jangwani contained stagnant, yellowish, evil-smelling water which had a salinity of 35 parts of chlorine per 100,000 parts of water.

In the Nile at Pakwach and in the swamps near Dar-es-Salaam the larva of this variety was the only anopheline found among the *Pistia*. On two occasions (at Atura and Rhino Camp) it was associated with *A. nili*, while at other places it was found with *A. coustani*.

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THE TRYPANOCIDAL ACTIVITY *IN VITRO* OF AROMATIC THIOARSINITES AND NEOARSPHENAMINE*

BY

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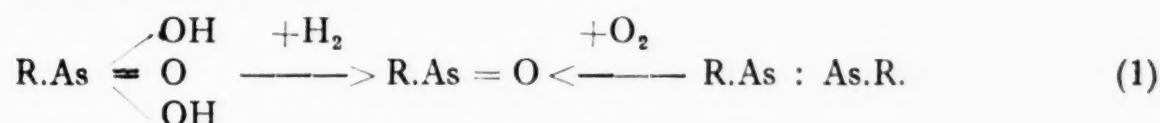
(Received for publication 19 August, 1937)

INTRODUCTION

In spite of the large number of arsenical compounds which have been prepared and tested for therapeutic activity in experimental trypanosomiasis, there is still no very clear conception of the exact form in which individual types of compound exert their lethal action. Ehrlich (1909) distinguished between pentavalent arsonic acids, on the one hand, and the trivalent arsenical compounds, arsenoxides and arseno-compounds, on the other. The arsonic acids he believed to be reduced by the tissues of the host to the corresponding arsenoxides before exerting their trypanocidal effect, while the trivalent arsenical compounds could act in the unchanged condition.

More recently, Warrington Yorke and his colleagues (1929-30) have investigated the trypanocidal activity *in vitro* of a number of different arsenical compounds. They found that, whereas pentavalent arsonic acids had very little trypanocidal activity *in vitro*, all compounds containing trivalent arsenic, whether arsenoxides of the type $R.AsO$, thioarsinites, $R.As \begin{smallmatrix} \swarrow SR' \\ \searrow SR' \end{smallmatrix}$ or arseno-compounds, $R.As = As.R$, were lethal to trypanosomes at very high dilutions. Like Ehrlich, they divide chemotherapeutic agents into two groups, namely, the intensely toxic trivalent arsenical compounds, the therapeutic effect of which is ascribed to the direct trypanocidal action of the unchanged drugs, and the weakly toxic arsonic acids.

Voegtlin and his colleagues (1923) have suggested that all arsenical compounds, whether pentavalent arsonic acids or trivalent arseno-compounds, are reduced or oxidized, respectively, to the corresponding arsenoxides by the tissues of the host, thus:



It is this arsenoxide which they believe to exert the trypanocidal effect. Support for the theory was supplied by the fact that, of the three types of substance,

* The work described in this paper formed part of a thesis approved for the degree of Doctor of Philosophy in the University of London.

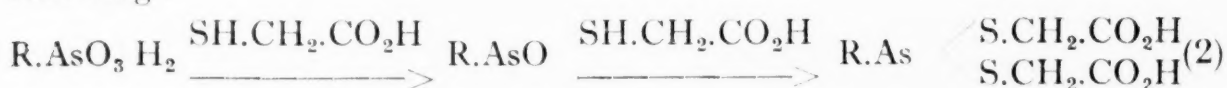
pentavalent arsonic acids and trivalent arseno-compounds showed a definite latent period between intravenous injection into a trypanosome-infected animal and the disappearance of the parasites from the blood-stream. Arsenoxides, on the other hand, caused a rapid disappearance of trypanosomes from the peripheral blood immediately after injection.

PRESENT INVESTIGATION

A series of stable chemical substances, formed by the condensation of aromatic arsenoxides with sulphhydryl compounds, have been prepared by King and his colleagues (Cohen, King and Strangeways, 1931, 1932) and their therapeutic value in curing *Trypanosoma equiperdum* infections in mice examined. It was found that in nearly all cases these compounds were less toxic to the host than their content of arsenoxide would require, while, in many cases, a substance with a high therapeutic index was produced by the condensation with a sulphhydryl compound of an oxide with a low therapeutic index. It was thought that an investigation of the trypanocidal activity *in vitro* of compounds of this type might throw some light on the exact form in which they exert their lethal effect. It might be supposed either (1) that they act without chemical change in structure, or (2) that they must first undergo hydrolytic fission with the production of the intensely active arsenoxide. The methods used for this study have been applied to neoarsphenamine, which, again, might be supposed to exert its curative action either (1) in the unchanged condition or (2) after oxidation to the active arsenoxide, R.AsO.

TRYPANOCIDAL ACTIVITY OF THIOARSINITES

The significance of thioarsinites as possible chemotherapeutic agents was first foreshadowed in 1908 when Friedberger (1908) found that p-aminophenyl-arsonic acid, mixed with thioglycollic acid, became more toxic to mice and also trypanocidally active *in vitro*. Berthelm (quoted by Roehl, 1909) showed, by analogy with arsenious acid, that this was due to the production of arsenoxide by reduction, with subsequent formation of thioarsinite, as shown in the following :



Much later, Voegtlin, Dyer and Leonard (1923) prepared the compound di(carboxymethyl)3-amino-4-hydroxyphenyl thioarsinite by heating together salvarsan oxide and thiolacetic acid. This compound showed a definite delay in trypanocidal action as compared with the parent arsenoxide.

In the present investigation the trypanocidal activities *in vitro* have been studied of thioarsinites produced by the condensation of arsenoxides with two naturally occurring sulphhydryl compounds, glutathione and cysteine.

METHOD

The method employed has been that devised by Yorke, Adams and Murgatroyd (1929) for the maintenance of trypanosomes alive in serum at 37° C. for a number of hours, and the investigation of the directly lethal action of various drugs upon them. Sheep serum was used in all experiments. In many cases Tyrode solution containing 0.2 per cent. glucose was substituted for the glucose-Ringer solution.

A strain of *Trypanosoma equiperdum* passaged through rats was used for the investigation. An infected rat was killed with coal gas and bled from the heart into citrate saline. The citrated blood was centrifuged lightly and the supernatant fluid, containing trypanosomes and very few blood corpuscles, was diluted with glucose-Ringer or Tyrode to the required richness. This was arranged so that one drop added to 0.5 c.cm. of medium gave a suspension of approximately 1,000 trypanosomes per cubic mm. Counts were made at hourly intervals by the removal to a Thoma-Zeiss haemocytometer of a drop of fluid from appropriate tubes in a series. Using a 1/6 inch objective and a no. 1 eyepiece, all moving trypanosomes in 100 small squares of the haemocytometer chamber were counted. Experiments were continued for 6 hours, the concentration of drug killing all the trypanosomes in a suspension being found for each hour.

Table I gives detailed results of an experiment with an arsenoxide, benzamide-p-arsenoxide. This experiment was designed to show that the

TABLE I

Concentration of drug	1. Trypanosomes added immediately									2. Trypanosomes added after 6 hours					
	1/35*	2/15	3/0	4/15	5/0	6/0	8/15	10/15	11/45	1/0	2/0	3/0	4/0	5/0	6/0
1: 400,000	0	—	—	—	—	—	—	—	—	0	—	—	—	—	—
1: 800,000	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1: 1,600,000	0	—	—	—	—	—	—	—	—	0	—	—	—	—	—
1: 3,200,000	—	1	0	—	—	—	—	—	—	—	5	0	—	—	—
1: 6,400,000	21	18	2	0	—	—	—	—	—	37	10	0	—	—	—
1: 12,800,000	—	23	30	8	1	0	—	—	—	—	30	8	0	0	—
1: 25,600,000	41	—	—	24	17	3	0	—	—	48	—	20	5	1	0
1: 51,200,000	—	—	—	35	33	39	29	7	2	—	—	37	43	40	28
1: 102,400,000	35	—	—	—	—	—	47	40	30	40	—	—	—	—	45
1: 204,800,000	—	—	—	—	—	—	—	30	31	—	—	—	—	—	—
Control—nutrient medium alone	41	—	—	34	—	—	—	—	42	44	—	—	—	42	—

*Time: 1/35 = 1 hour, 35 minutes; etc.

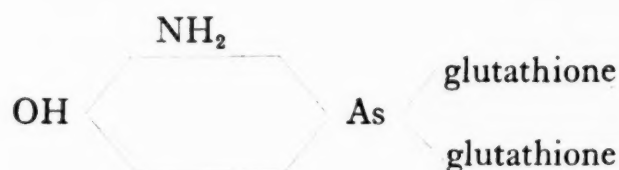
toxicity of the compound did not change during the course of an experiment. Two series of dilutions were prepared. Trypanosomes were added to the first immediately and to the second after it had been standing in the water-bath at 37° C. for 6 hours.

The above table is typical of those from which later tables were compiled.

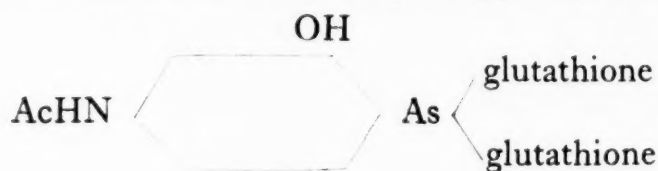
COMPOUNDS TESTED

The three thioarsinites examined for their toxicity to trypanosomes *in vitro* were :

(1) Diglutathionyl-3-amino-4-hydroxyphenyl thioarsinite (salvarsan-oxide-glutathione)



(2) Diglutathionyl-4-acetamino-2-hydroxyphenyl thioarsinite (K.352)



(3) Dicysteinyi-benzamide-p-thioarsinite (K.324)



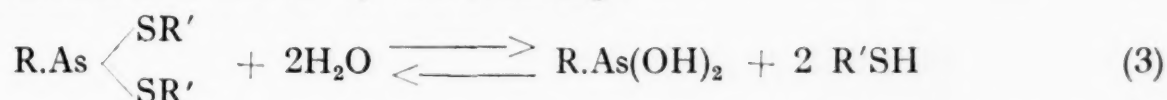
Table II gives the results of typical experiments on the toxicity of each compound. These results are not strictly comparable, since they were not all obtained on the same day.

TABLE II

Compound	Concentration of compound killing all trypanosomes in :—					
	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours
Salvarsan oxide-glutathione	1 : 1,600,000	1 : 1,600,000	1 : 3,200,000	1 : 3,200,000	1 : 6,400,000	1 : 12,800,000
K.352	1 : 400,000	1 : 800,000	1 : 1,600,000	1 : 3,200,000	1 : 3,200,000	1 : 6,400,000
K.324	1 : 3,200,000	—	1 : 6,400,000	1 : 12,800,000	1 : 12,800,000	1 : 25,600,000

The high dilutions at which the three substances have a trypanocidal effect is noteworthy, and they are comparable with the results obtained by Yorke and his collaborators with trivalent arsenicals.

From chemical experiments (Cohen, King and Strangeways, 1931a) it is known that thioarsinites are readily hydrolyzed into free thiol compounds and arsenoxides, as shown by the following:



The right-hand side is favoured by alkaline solutions, since in sodium hydroxide solution there is complete dissociation, whilst the left-hand side is favoured by neutrality, since a solution of thioarsinite in sodium bicarbonate solution gives a negligible nitroprusside reaction for free thiol groups. The left-hand side is also favoured by excess of the sulphhydryl compound, and, conversely, a very dilute aqueous solution of the thioarsinite, where the active mass of the molecules of water preponderate, should favour hydrolysis to arsenoxide, or its hydrate, and free sulphhydryl compound.

The results with K.324 shown in Table III, where a comparison, showing identity, is made between the action of the thioarsinite dissolved in sodium bicarbonate and its action dissolved in sodium hydroxide, suggest that the action in the bicarbonate solution is due to free arsenoxide produced by the high dilutions employed.

TABLE III

K.324	Concentration of K.324 killing all trypanosomes in:—				
	1 hour	3 hours	4 hours	5 hours	6 hours
(1) In NaHCO_3	1 : 3,200,000	1 : 6,400,000	1 : 12,800,000	1 : 12,800,000	1 : 25,600,000
(2) In NaOH	1 : 3,200,000	1 : 6,400,000	1 : 6,400,000	1 : 12,800,000	1 : 12,800,000

Another experiment designed to investigate this point was made with the compound of salvarsan oxide and glutathione.

It is clear that, if the lethal action of the compound for trypanosomes *in vitro* is due to free salvarsan oxide, an agreement between the toxicity of equimolecular solutions of the oxide and its glutathione compound should be obtained, provided that the hydrolysis of the latter is complete, since the toxicity of glutathione is negligible when compared with arsenoxide.

The molecular weight of the dichloride hydrochloride of salvarsan oxide is 290 and that of the glutathione compound 795. A five-thousandth molar

solution of the former was made by dissolving 0.06 gm. in glucose-Ringer solution, neutralizing with saturated sodium bicarbonate and making up to 10 c.cm. with glucose-Ringer. One c.cm. of this solution was then diluted to 100 c.cm. with glucose-Ringer. Similarly 0.16 gm. of the glutathione compound was neutralized and made up to 10 c.cm., 1 c.cm. then being diluted to 100 c.cm. Two series of tubes were thus obtained, the first containing dilutions of salvarsan oxide and the second of salvarsan oxide-glutathione, both ranging from M/10,000 to M/10,240,000. The results of two experiments made on consecutive days are given in Table IV.

TABLE IV

Compound	Concentration in which all trypanosomes were killed in :—					
	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours
Salvarsan oxide	M/160,000	M/640,000	M/1,280,000	M/2,560,000	M/2,560,000	M/5,120,000
Salvarsan oxide-glutathione	M/320,000	M/640,000	M/640,000	M/2,560,000	M/2,560,000	M/5,120,000
Salvarsan oxide	M/160,000	M/320,000	M/640,000	M/640,000	—	M/2,560,000*
Salvarsan oxide-glutathione	M/160,000	M/320,000	M/640,000	M/1,280,000	—	M/5,120,000†

*M/5,120,000 1 trypanosome seen ; M/10,240,000 13 trypanosomes seen.

†M/10,240,000 11 trypanosomes seen.

Controls (nutrient medium alone) 48 and 49 trypanosomes seen.

The correspondence between the two sets of figures is sufficiently close to indicate that the thioarsinite of salvarsan oxide and glutathione exerts its toxic action *in vitro* in virtue of the oxide it yields on hydrolysis.

EFFECT OF EXCESS OF GLUTATHIONE

A further experiment which suggested itself was to find out whether the toxic action of the thiol compound could be prevented by the presence of excess of glutathione, as shown by Voegtlin, Dyer and Leonard (1923) to be the case with the parent oxide. Table V gives the somewhat surprising results obtained in two experiments on different days with salvarsan oxide-glutathione and with salvarsan oxide.

Three series of tubes were prepared in each experiment. The first contained increasing dilutions of the arsenical compound alone, the second contained the same dilutions of the arsenical with 10 molecular equivalents of glutathione, while the third contained dilutions of the arsenical with 2,000 molecular equivalents of glutathione.

There is clearly a discrepancy between the results given in Table V, showing the absence of any inhibitory action by glutathione, and those obtained by Voegtlin, Dyer and Leonard, who found that the toxic action of salvarsan oxide was completely inhibited by the presence of 10 molecular equivalents of glutathione.

TABLE V

Compound	Concentration of arsenicals in which no trypanosomes were seen in :—					
	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours
Salvarsan oxide-gluta- thione—						
(1) Alone	1 : <800,000	—	1 : 800,000	1 : 1,600,000	1 : 3,200,000	1 : 3,200,000
(2) + 10 mols. GSH*	1 : <800,000	—	1 : 800,000	1 : 1,600,000	1 : 3,200,000	1 : 3,200,000
(3) + 2,000 " "	1 : <800,000	—	1 : 800,000	1 : 1,600,000	1 : 3,200,000	1 : 3,200,000
Salvarsan oxide—						
(1) Alone	1 : 800,000	1 : 800,000	1 : 800,000	1 : 1,600,000	1 : 1,600,000	1 : 3,200,000
(2) + 10 mols. GSH	1 : <800,000	—	1 : 800,000	1 : 1,600,000	1 : 1,600,000	1 : 3,200,000
(3) + 2,000 " "	1 : <800,000	—	1 : 800,000	1 : 1,600,000	1 : 3,200,000	1 : 3,200,000

GSH* = glutathione.

A number of experiments were made to investigate this discrepancy. It was found that, using the dilutions of arsenoxide given by Voegtlin, Dyer and Leonard (1923) in the table on page 1889 of their communication, namely, M/200–M/1,000 solutions of salvarsan oxide, complete inhibition could be obtained over a number of hours by the addition of 10 molecular equivalents of glutathione. When, however, the solutions became more dilute, protection became less complete, until, at the higher dilutions, it was not apparent at all. Table VI gives the results obtained in an experiment made at 37° C. in the nutrient medium used for other experiments.

An M/50 solution of salvarsan oxide was made by dissolving 0.06 gm. of 3-amino-4-hydroxyphenyl arsine dichloride hydrochloride in a small amount of distilled water, neutralizing with normal sodium hydroxide and making up to 10 c.cm., with Tyrode solution. Similarly, an M/5 solution of glutathione was made by suspending 0.31 gm. in a little distilled water, neutralizing with normal sodium hydroxide and making up to 5 c.cm. with Tyrode. The mixture of 1 c.cm. of each of these solutions gave a solution of M/100 salvarsan oxide in the presence of M/10 glutathione. Dilutions of the mixture of salvarsan oxide and glutathione, of salvarsan oxide alone, and of glutathione alone, were made in the usual way, but instead of the addition of a drop of trypanosome

suspension to each tube, as in previous experiments, an equal volume (0.5 c.cm.) of a fairly rich suspension of trypanosomes was added, and the results, obtained by microscopical examination of drops removed at intervals, were recorded.

It will be seen from Table VI that, whereas in the higher concentrations a definite reduction in the toxicity of salvarsan oxide is produced by the presence of 10 molecules excess of glutathione, there is no such reduction in the lower concentrations. In these latter the toxicity of salvarsan oxide is the same, whether alone or in the presence of glutathione.

It was shown, further, (1) that there was no difference in the results obtained in nutrient medium containing serum and those obtained using glucose-Ringer solution alone; (2) that the protective action of glutathione was more pronounced at room-temperature than at 37° C. It was, of course, at room-temperature that Voegtlin, Dyer and Leonard made their *in vitro* experiments.

Very similar results were obtained if preformed salvarsan oxide-glutathione was used instead of salvarsan oxide. In one experiment a comparison was made of the effect of glutathione on the activity of equimolecular dilutions of salvarsan oxide and its glutathione compounds. In this experiment 10 molecular equivalents of glutathione were present in the solution of salvarsan oxide, while 8 equivalents were present in that of the glutathione compound. Identical results were obtained in each series of dilutions.

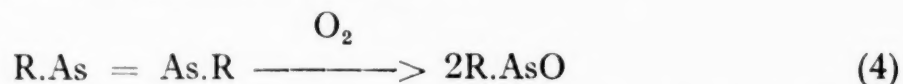
DISCUSSION OF RESULTS

The foregoing account demonstrates fairly clearly that the trypanocidal activity *in vitro* of thioarsinites is due to their content of arsenoxide. In strong solutions the toxic action both of salvarsan oxide and of its glutathione compound can be inhibited by the presence of excess of glutathione which favours the formation of the thioarsinite (see equation (3)). In more dilute solutions the great affinity of arsenic for sulphur is overwhelmed by the great preponderance of water molecules which bring about hydrolysis of the thioarsinite to liberate the toxic arsenoxide.

TRYPANOCIDAL ACTION OF NEOARSPHENAMINE

That neoarsphenamine is lethal to trypanosomes *in vitro* in low concentrations is now well known. This has been amply verified by the work of Yorke and Murgatroyd (1930), using the technique which they have developed for investigating the trypanocidal activity of a number of substances at 37° C. Little is known, however, of the exact mechanism of its lethal action.

In Voegtlin's view (1920) arseno-compounds exert their lethal action *in vivo* after oxidation to the arsenoxide as follows:



Support for this theory was provided by experiments showing that there was a definite latent period after the injection of an arseno-compound before the

TABLE VI

Dilution salvarsan oxide	Dilution GSH	Salvarsan oxide + 10 mols. GSH						Salvarsan oxide alone						GSH alone					
		0/5*	0/15	0/30	1/0	1/30	3/0	4/0	0/5	0/15	0/30	1/0	1/30	3/0	4/0	0/15	1/0	3/0	4/0
—	M/10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	L	—	—	—
M/200	M/20	L	—	L	—	L	S/sl	dis	—	—	—	—	—	—	—	—	L	—	L
M/400	M/40	—	L	—	L	L/sl	dis	—	dis	—	—	—	—	—	—	L	—	L	—
M/800	M/80	L	—	L	L	dis	—	—	dis	—	—	—	—	—	—	—	L	—	L
M/1,600	M/160	—	L	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
M/3,200	M/320	L	—	dis	—	—	—	—	dis	—	—	—	—	—	—	—	L	—	L
M/6,400	M/640	—	sl	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
M/12,800	M/1,280	L	—	dis	—	—	—	—	S/sl	dis	—	—	—	—	—	—	L	—	L
M/25,600	M/2,560	—	sl	—	—	—	—	—	—	dis	dis	—	—	—	—	—	L	—	L
M/51,200	M/5,120	L	—	S	—	—	—	—	L	S/sl	dis	—	—	—	—	—	L	—	L
M/102,400	M/10,240	—	L/sl	—	dis/sl	dis	—	—	—	sl	dis/sl	dis	dis	—	—	—	—	—	—
M/204,800	M/20,480	L	—	L	sl/S	dis/sl	dis	—	L	L	sl/S	dis/sl	dis	dis	—	—	—	—	—
M/409,600	M/40,960	—	L	—	L/sl	S/sl	dis	—	—	L	L/sl	S/sl	dis/sl	dis	L	—	L	—	—
Controls— (nutrient medium alone)		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	L	—	L

S = still; L = lively; sl = sluggish; dis = disintegrating.

* Time: 0/5 = 0 hours, 5 minutes; etc.

trypanosomes disappeared from the blood of an infected animal. No such latent period occurred when the corresponding arsenoxide was injected.

Simić (1923), in investigating the difference between the toxicity of neoarsphenamine for spirochaetes and for trypanosomes, believed that its more rapid lethal action on the latter, both *in vivo* and *in vitro*, was due to the fact that neoarsphenamine could be adsorbed in the unchanged condition by trypanosomes, whereas oxidation to the arsenoxide was necessary before it could enter the spirochaetes. He suggested, further, that, although the oxidized form of neosalvarsan was very much more toxic to trypanosomes than was the unchanged product, this latter had a trypanocidal action.

Papamarku (1927) found that, if a mixture of trypanosomes and spirochaetes was treated with different concentrations of neoarsphenamine, the trypanosomes were killed in 2 hours by a dilution of 1 in 10 millions, while the spirochaetes remained healthy for a number of hours in much higher concentrations. These, too, however, were killed by dilutions of 1 in 500,000 in 24 hours and 1 in 20 millions in 48 hours. Considering his own and Simić's results, Papamarku concluded that the observed difference in time of reaction was due to the fact that the trypanosomes themselves were able to oxidize the arseno-compound to the toxic oxide.

It was thought that some light might be thrown on this question by investigating the effect of the presence of excess of glutathione on the toxicity of neoarsphenamine to trypanosomes *in vitro*. If it is the oxide which is responsible for the toxicity of arseno-compounds, then it should be possible to protect trypanosomes *in vitro* against their lethal action by this addition of excess of glutathione.

Preliminary experiments on the toxicity of neoarsphenamine for trypanosomes gave results similar to those obtained by Yorke and Murgatroyd (1930). Table VII summarizes the results of an experiment to compare the trypanocidal activity of four samples of neoarsphenamine. Three of these, A, B and C, were manufacturers' products, and one, D, a sample prepared by Dyke and King (1934).

TABLE VII

Sample	Concentration in which all trypanosomes were dead in :—					
	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours
A	1 : 400,000	1 : 1,600,000	1 : 3,200,000	1 : 6,400,000	1 : 12,800,000	1 : 12,800,000
B	1 : 400,000	1 : 1,600,000	1 : 3,200,000	1 : 3,200,000	1 : 6,400,000	1 : 12,800,000
C	1 : 400,000	1 : 1,600,000	1 : 1,600,000	1 : 3,200,000	1 : 6,400,000	1 : 6,400,000
D	1 : 400,000	1 : 1,600,000	1 : 3,200,000	1 : 6,400,000	1 : 12,800,000	1 : 12,800,000

A, B and C = Manufacturers' samples.

D = Sample of Dyke and King (1934).

There is remarkably good agreement between the trypanocidal activities *in vitro* of these neoarsphenamines, although Dyke and King (1934) found differences in their chemical properties.

Table VIII gives the results of an experiment to show that the toxicity of neoarsphenamine is not altered during the course of an experiment. Two series of dilutions were made. Trypanosome suspension was added to series A immediately, while the tubes of series B were kept in the water-bath at 37° C. for 6 hours before the addition of trypanosomes.

TABLE VIII

Concentration in which all trypanosomes were dead in :—								
	10 minutes	20 minutes	30 minutes	1 hour	2 hours	3 hours	4 hours	6 hours
A	1 : 200,000	1 : 200,000	1 : 200,000	1 : 200,000	1 : 400,000	1 : 1,600,000	1 : 1,600,000	1 : 3,200,000
B	1 : 200,000	1 : 200,000	1 : 200,000	1 : 200,000	1 : 400,000	—	—	—

A = Neoarsphenamine dilutions freshly prepared.

B = " " " incubated 6 hours at 37° C.

The lethal concentrations of drug were somewhat higher than those given in Table VII, but the experiment illustrates the stability of the neoarsphenamine solution as far as trypanocidal activity is concerned. This is in agreement with the results of Yorke and Murgatroyd (1930), who found that high dilutions of neoarsphenamine 'whether previously oxidized or not' were lethal to trypanosomes when examined over a number of hours.

Experiments were now made to find the effect of the presence of excess of glutathione on the trypanocidal activity of neoarsphenamine. Since no accurate estimate of the molecular weight of neoarsphenamine can be made, the preparations were calculated on the basis of the arsenic content of the product. Table IX gives the results of an experiment with a neoarsphenamine containing 20 per cent. of arsenic.

An M/50 solution of neoarsphenamine (0.074 gm. in 10 c.cm. solution) and an M/5 solution of glutathione (0.31 gm. neutralized with N.NaOH and made up to 5 c.cm.) were prepared in Tyrode. The mixture of 1 c.cm. of each solution gave an M/100 solution of neoarsphenamine in the presence of M/10 glutathione. Dilutions of this mixed solution, of glutathione alone and of neoarsphenamine alone were made in the usual way, and the experiment started by the addition of 0.5 c.cm. of trypanosome suspension to each tube of the three series. The death of the trypanosomes was followed by the microscopical

TABLE IX

Concentra- tion of NS	Concentra- tion of GSH	Neoarsphenamine + 10 mols. GSH								Neoarsphenamine alone				GSH alone					
		0/10†	0/30	1/0	2/0	3/0	4/0	5/0	6/0	0/10	0/30	1/0	2/0	3/0	4/0	5/0	6/0		
M/200*	M/20	—	—	—	—	—	—	—	—	S	—	—	—	L	—	—	—		
M/400	M/40	L	—	L	—	L	L	L	L	—	—	—	—	L	—	L	L		
M/800	M/80	—	L	—	L	L	L	L	S/sl	S	—	—	—	L	—	L	L		
M/1,600	M/160	L	—	L	—	L	L	S	—	—	—	—	—	L	—	L	L		
M/3,200	M/320	—	L	—	L	dis	dis	—	—	S	—	—	—	L	—	L	L		
M/6,400	M/640	L	—	L	—	—	—	—	—	—	—	—	—	L	—	L	L		
M/12,800	M/1,280	—	L	—	dis	—	—	—	—	S	—	—	—	L	—	L	L		
M/25,600	M/2,560	L	—	S	—	—	—	—	—	—	S	—	—	L	—	L	L		
M/51,200	M/5,120	—	L	—	dis	—	—	—	—	L	S	—	—	L	—	L	L		
M/102,400	M/10,240	L	—	sl	—	—	—	—	—	—	L/sl	S/sl	dis	—	—	—	—		
M/204,800	M/20,480	—	L	—	S	—	—	—	—	L	—	L	L	—	—	L	—		
Controls— nutrient medium alone		—	L	—	L	—	—	—	—	—	—	—	—	—	L	—	L		

L = lively; sl = sluggish; S = still; dis = disintegrating.

*Dilutions of neoarsphenamine calculated on arsenic content.

†Time: 0/10 = 0 hours, 10 minutes; etc.

examination of drops removed at intervals. The trypanocidal activity of neoarsphenamine is, therefore, prevented by the presence of excess of glutathione in the same way as is that of an arsenoxide. Glutathione in its reduced form will not react chemically with an arseno-compound; it is, therefore, reasonable to assume that the directly toxic substance is an arsenoxide formed by oxidation of the neoarsphenamine. This production of arsenoxide does not appear to take place by aerial oxidation, as is proved by Table VIII, and the only way to account for the result is to suppose that the neoarsphenamine is taken up by the trypanosomes and becomes oxidized either on the surface or within the cell by the normal oxidation processes operating in the trypanosomes. In the more concentrated solutions the large excess of glutathione combines with the arsenoxide formed by the oxidation of the arseno-group and thus inhibits the lethal action over the period studied; in the more dilute solutions the excess of glutathione is insufficient to counterbalance the hydrolytic action of the preponderating water molecules on the thioarsinites formed. This hypothesis can also be advanced to explain the results of Moncorps and Bohnstedt (1934). These authors found that concentrations of glutathione of 1 in 100 to 1 in 500 reduced the trypanocidal activity of a 1 in 7,000 dilution of neosalvarsan both *in vivo* and *in vitro*.

These *in vitro* experiments with neoarsphenamine lend experimental support, therefore, to the hypothesis advanced by Papamarku on its mode of action on trypanosomes. The experiments also throw a new light on the possible mode of action of neoarsphenamine *in vivo*. After intravenous injection into an infected mouse the arseno-compound is distributed all over the body and adsorbed as such by the trypanosomes as well as by the cells of the host. The oxidative processes within the trypanosome itself will, then, produce the lethal arsenoxide. Support for this conception is also supplied by the experiments of Gonder (1912), Castelli (1913) and Simić (1923), which showed that, both *in vitro* and *in vivo* (Simić), trypanosomes which had been in contact with neoarsphenamine for a short time (5 to 30 minutes) retained their motility but would no longer infect mice.

It is possible that the production of arsenoxide by the cells of the host may also play a part in the therapeutic effect of neoarsphenamine. Simić has, indeed, suggested that in spirochaetal infections the greater therapeutic efficiency and the higher toxicity of neoarsphenamine to the host on subcutaneous injection, when compared with intravenous injection, is partly due to the more rapid production of arsenoxide in the former instance, although more rapid excretion in the latter is also a factor.

MISCELLANEOUS CONTROL EXPERIMENTS

The following experiments were designed to show that the results obtained with glutathione could not be attributed to some non-specific protection of the trypanosomes against the arsenicals by the high concentrations employed.

An experiment which can be included here demonstrates that the protective action of cysteine against the toxicity of neoarsphenamine was similar to that obtained with glutathione. Cysteine, the sulphhydryl-containing component of the tripeptide glutathione, was found by Voegtlin, Dyer and Leonard (1923) to have the same effect as glutathione. Table X gives the results obtained.

A 1 in 100 solution of neoarsphenamine (0.1 gm. in 10 c.cm.) and an approximately 1 in 24 solution of cysteine (0.42 gm. neutralized with N.NaOH and made up to 10 c.cm.) were made in Tyrode. The mixture of 1 c.cm. of each of these solutions gave a 1 in 200 solution of neoarsphenamine in the presence of 10 molecular proportions of cysteine. Dilutions were made in the usual manner, and the experiment started by the addition of an equal volume (0.5 c.cm.) of trypanosome suspension to each tube. The death of the trypanosomes was followed by microscopical examination of drops removed from each tube at intervals.

The protective action of cysteine for trypanosomes, against the lethal action of neoarsphenamine, is therefore similar to that obtained with glutathione.

The possibility that glutathione afforded some non-specific protection to trypanosomes against toxic substances was tested by trying the effect of excess of glutathione on substances other than aromatic arsenicals. The two substances which suggested themselves were tartar emetic and acriflavine.

1. *Tartar emetic.* $\begin{matrix} \text{CH}_2\text{COOK} \\ \text{CH}_2\text{COOSbO}_3 \end{matrix}$ Molecular weight = 334. Table XI gives

the results of an experiment testing the protective power of glutathione against the trypanocidal activity of tartar emetic. The glutathione was present in the same proportions in each tube, namely, 10 molecules to 1 molecule of tartar emetic.

An M/50 solution of tartar emetic (0.07 gm. in 10 c.cm.) and an M/5 solution of glutathione (0.62 gm. neutralized with N.NaOH and made up to 10 c.cm.) were made in Tyrode solution. The mixture of 1 c.cm. of each of these solutions gave M/100 tartar emetic in the presence of M/10 glutathione. Dilutions of the mixed solution and of the separate solutions were made in the usual way, and the experiment started by the addition of an equal volume (0.5 c.cm.) of trypanosome suspension to each tube. The death of the trypanosomes was followed by the examination of drops removed at intervals.

The addition of excess of glutathione to a solution of tartar emetic does not, therefore, reduce its toxicity for trypanosomes.

2. *Acriflavine.* Molecular weight = 296.

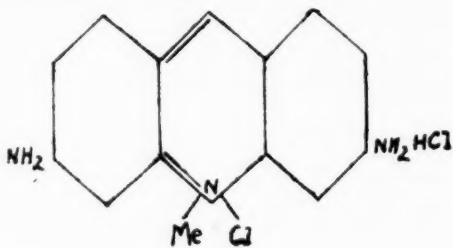


TABLE X

Concentra- tion of NS	Concentra- tion of cysteine	NS* + 10 mols. cysteine						NS alone			Cysteine alone							
		0/15	0/35	1/0	2/0	3/0	4/0	5/0	0/15	0/40	1/0	0/15	0/40	2/0	3/0	4/0	5/0	6/0
—	1:96	—	—	—	—	—	—	—	—	—	—	L	—	L	—	S/sl	dis	—
1:800	1:192	L	—	L	L	L/sl	S/sl	dis	—	—	—	—	—	L	—	L/S	S/L	S/L
1:1,600	1:384	—	L	—	L	L	L/S	dis	S	—	—	L	—	L	—	L	L	—
1:3,200	1:768	L	—	L	L	L	L	dis	S	—	—	—	—	L	L	L	—	L
1:6,400	1:1,536	—	L	—	L	L	dis	—	—	—	—	—	—	L	L	L	L	—
1:12,800	1:3,072	L	—	L	dis	—	—	—	S	—	—	—	—	L	L	L	L	—
1:25,600	1:6,144	—	L	dis	—	—	—	—	—	—	—	—	—	L	—	—	L	—
1:51,200	1:12,288	S/sl	S	—	—	—	—	—	S	—	—	—	—	—	L	—	L	—
1:102,400	1:24,576	—	S	—	—	—	—	—	—	dis	—	S/sl	—	L	—	—	L	—
1:204,800	1:49,152	L	L/sl	dis	—	—	—	—	L	dis	—	S/sl	—	L	L	—	—	L
1:409,600	1:98,304	—	—	—	—	—	—	—	—	—	—	S/L	dis	—	—	—	—	—
Controls— nutrient medium alone		L	—	L	L	L	L	—	L	—	—	L	L	L	L	—	—	L

L = lively; sl = sluggish; S = still; dis = disintegrating.

*NS = neoarsphenamine.

When solutions of glutathione and acriflavine were mixed, a copious precipitate was formed which made any test impossible. A neutral solution of cysteine gave no such precipitate, and an experiment was therefore made with this compound, which had previously been shown to be effective in the case of neoarsphenamine (Table X).

TABLE XI

Concentration of T.E	Concentration of GSH	T.E + GSH			T.E alone			GSH alone		
		0/10*	0/25	1/10	0/10	0/25	1/10	0/10	0/25	1/10
M/200	M/20	—	—	—	dis	—	—	L	—	L
M/400	M/40	dis	—	—	—	—	—	—	L	—
M/800	M/80	—	—	—	dis	—	—	L	—	L
M/1,600	M/160	dis	—	—	—	—	—	—	L	—
M/3,200	M/320	—	—	—	dis	—	—	L	—	L
M/6,400	M/640	dis	—	—	—	—	—	—	L	—
M/12,800	M/1,280	—	dis	—	dis/sl	dis	—	L	—	L
M/25,600	M/2,560	L	dis/sl	dis	—	dis/sl	dis	—	L	—
M/51,200	M/5,120	—	L	dis	L	L	dis	L	—	L
M/102,400	M/10,240	L	L	sl	—	L	sl	—	L	—
M/204,800	M/20,480	—	L	L	—	—	—	—	—	—
<i>Controls—</i> nutrient medium alone		L	L	L	L	L	L	L	L	L

L = lively; sl = sluggish; dis = disintegrating.

T.E. = tartar emetic. GSH = glutathione.

* Time: 0/10 = 0 hours, 10 minutes; etc.

An M/50 solution of acriflavine (0.06 gm. in 10 c.cm.) and an M/5 solution of cysteine (0.31 gm. neutralized with N.NaOH and made up to 10 c.cm.) were made in Tyrode solution. The mixture of 1 c.cm. of each of these solutions gave an M/100 solution of acriflavine in the presence of M/10 cysteine. Dilutions of the mixed solution and of acriflavine alone were made in the usual way, and the experiment started by the addition of an equal volume, 0.5 c.cm., of trypanosome suspension to each tube. Death of the trypanosomes was followed by microscopical examination of drops removed at intervals. The results are given in Table XII.

This failure to obtain protection against the toxic action of acriflavine by a sulphydryl compound is in agreement with the results obtained by Pedlow and Reiner (1935), who were unable to find any depression in the trypanocidal activity of acriflavine by the addition of thioglycollic acid.

TABLE XII

Concentration of acriflavine	Concentration of cysteine	Acriflavine + cysteine				Acriflavine alone			
		0/10*	0/30	1/0	3/35	0/10	0/30	1/0	3/35
M/400	M/40	S	—	—	—	S	—	—	—
M/800	M/80	—	—	—	—	—	—	—	—
M/1,600	M/160	S	—	—	—	S	—	—	—
M/3,200	M/320	—	—	—	—	—	—	—	—
M/6,400	M/640	S	—	—	—	S	—	—	—
M/12,800	M/1,280	—	—	—	—	—	—	—	—
M/25,600	M/2,560	S	—	—	—	S	—	—	—
M/51,200	M/5,120	—	S/sl	S	—	—	S/sl	S	—
M/102,400	M/10,240	L	L/sl	sl	S	L	L/sl	sl	S
M/204,800	M/20,480	—	L	L	sl	—	L	L	sl
Controls—nutrient medium alone		L	L	—	L	L	L	L	L

L = lively ; sl = sluggish ; S = still.

* Time : 0/10 = 0 hours, 10 minutes ; etc.

CONCLUSIONS AND SUMMARY

The conclusions which can be arrived at from the results obtained in the present communication can be summarized briefly as follows.

Thioarsinites have a toxicity for trypanosomes *in vitro* in high dilution. That this toxicity is due to the hydrolysis of the compound in aqueous solution with liberation of the highly trypanocidal arsenoxide is shown by :

1. The identity in the lethal activity of equimolecular concentrations of thioarsinite and its parent oxide.

2. The inhibition of the lethal action both of an arsenoxide and a thioarsinite in strong solution by the addition of 10 molecules excess of glutathione, and the failure to obtain protection in higher dilutions owing to the preponderating influence of water molecules.

Now, thioarsinites have been shown to be more effective as therapeutic agents in experimental trypanosomiasis than the corresponding arsenoxides (Cohen, King and Strangeways, 1931a). Since, as is shown above, the directly trypanocidal agent of a thioarsinite is the arsenoxide, this requires some explanation.

Thioarsinites are readily obtained in solution as neutral sodium salts. At the relatively high concentrations (1 in 1,000 to 1 in 100) in which these solutions are introduced into the blood-stream, there is very little hydrolysis to the arsenoxide and sulphydryl components, as shown by a negligible nitroprusside reaction. After intravenous injection and consequent dilution of

thioarsinite, there will be some hydrolysis with liberation of free arsenoxide. This partial hydrolysis is rapid, since the trypanosomes begin to disappear from the blood-stream within half an hour after injection; but it is not immediately complete, since, as has been pointed out above, the dose of a thioarsinite which can be tolerated is greater than would be expected from the calculated content of arsenoxide.

Neoarsphenamine has a trypanocidal action *in vitro* in low concentrations, although these are higher than those found for arsenoxides and thioarsinites. This toxicity is not increased by aerial oxidation during the course of an experiment. The addition of 10 molecules excess of glutathione to strong solutions of neoarsphenamine inhibits its lethal action, although a similar excess is without effect on the toxicity of high dilutions. This lends support to the suggestion that neoarsphenamine is adsorbed by the trypanosome and becomes oxidized, within the trypanosome or on its surface, to the highly trypanocidal arsenoxide. The inhibitory action of glutathione would, then, be the same as in the case of arsenoxides and thioarsinites.

ACKNOWLEDGEMENT. My thanks are due to Dr. H. King, F.R.S., who, throughout the research and in the preparation of this paper, has given me his constant advice and encouragement.

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OBSERVATIONS ON THE TRYPANOCIDAL ACTION *IN VITRO* OF SOLUTIONS OF GLUTATHIONE AND ASCORBIC ACID

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(*Received for publication 19 August, 1937*)

During the course of an investigation of the trypanocidal activity of thioarsinites and of neoarsphenamine (Strangeways, 1937), a phenomenon was observed which is the subject of the present communication. The experimental method, devised by Yorke, Adams and Murgatroyd (1929), and the constitution of the nutrient medium have already been described.

It was found that different concentrations of glutathione in Tyrode solution containing an equal volume of serum which had been heated for 1 hour at 56° C., or in Tyrode alone, exerted a definite lethal action on suspensions of trypanosomes containing about 1,000 organisms per cubic mm. Death of the trypanosomes in such a medium did not occur earliest in the highest or the lowest concentration of glutathione, but in an intermediate zone of concentrations. When, on the other hand, fresh unheated serum was used for the medium, the trypanosomes usually remained healthy in all but the highest concentrations of glutathione. Occasionally a zone of toxicity was observed when different concentrations of glutathione were tried, even in a medium containing unheated serum; but this effect was never so pronounced as that shown in the presence of heated serum.

Table I gives the results of one experiment, typical of many, illustrating this phenomenon.

Sheep serum was obtained on the day before the experiment. Some of this was inactivated for 1 hour at 56° C., while the rest was unheated. An M/2.5 solution of glutathione was prepared by neutralizing 0.62 gm. with normal sodium hydroxide, and making up to 5 c.cm. with glucose-Ringer solution. Two series of dilutions of this solution were prepared—series A, in nutrient medium containing unheated serum, and series B, in medium containing serum which had been heated for 1 hour at 56° C. The experiment was started by the addition of 1 drop of trypanosome suspension to each tube of each series. This suspension was made by diluting in glucose-Ringer the citrated blood of a rat infected with a strain of *Trypanosoma equiperdum*. At intervals, small samples of fluid were removed from representative tubes of each series. These samples were placed on Thoma-Zeiss haemocytometer slides and the number of trypanosomes in 100 small squares of the counting chamber recorded. These counts are given in Table I.

TABLE I

Dilutions of glutathione	Series A					Series B				
	Unheated serum					Serum heated 1 hour at 56° C.				
	1/0*	2/15	3/10	4/40	6/5	1/30	2/0	3/25	5/0	6/40
M/5	14	5	—	3	2	12	7	—	2	0
M/10	15	—	11	3	5	7	—	4	1	1
M/20	13	12	—	13	13	6	5	—	—	0
M/40	19	—	12	13	14	3	—	0	—	—
M/80	13	10	—	11	10	2	0	—	—	—
M/160	14	—	15	13	12	1	—	0	—	—
M/320	14	11	—	12	15	3	0	—	—	—
M/640	13	—	13	14	18	3	—	0	—	—
M/1,280	12	13	—	18	20	7	3	—	0	—
Controls—										
nutrient	12	10	—	14	14	12	16	—	14	15
medium alone	15	—	14	13	12	12	—	11	13	12

*Time: 1/0 = 1 hour; 2/15 = 2 hours, 15 minutes; etc.

The trypanosomes remained healthy in solutions of glutathione, in Tyrode + unheated serum, of concentration less than M/10 (Table I, series A); those in series B, where the serum had been heated for 1 hour at 56° C., died soon after the experiment started in the presence of glutathione M/40 down to M/640, and rather more slowly in stronger or weaker concentrations than these.

The results suggest that solutions of glutathione contain some substance which is toxic for trypanosomes. This toxic substance is either prevented from being formed, or destroyed as it is formed, by some heat-labile agent contained in samples of sheep serum. It seemed possible that hydrogen peroxide might be formed in solutions of an autoxidizable substance like glutathione. The protective heat-labile agent might then be catalase.

Table II gives the results of an experiment designed to show whether the addition of a preparation of catalase to inactivated serum reduced the toxicity of glutathione contained in this medium.

Tubes were prepared containing a series of concentrations of glutathione in Tyrode diluted with (A) inactivated serum, (B) unheated serum, and (C) inactivated serum to which catalase had been added. The solution of catalase used consisted of a sterile saline extract of acetone-dried horse liver. One c.cm. of this preparation was added to 4 c.cm. of the inactivated serum used for the

TABLE II

Dilutions of GSH*	Series A						Series B						Series C					
	Inactivated serum alone						Unheated serum alone						Inactivated serum + catalase					
	1/5†	2/15	3/10	4/10	5/10	6/20	1/15	2/25	3/20	4/20	5/20	6/25	0.55	2/0	3/0	4/0	5/0	6/0
M/20	22	—	8	2	0	—	15	—	6	—	5	3	14	—	11	—	3	0
M/40	—	17	—	3	1	—	—	20	—	20	—	8	—	19	—	10	—	0
M/80	18	—	2	0	—	—	21	—	20	—	10	12	15	—	14	—	5	1
M/160	—	13	0	—	—	—	—	20	—	11	—	20	—	21	—	21	—	24
M/320	2	—	0	—	—	—	19	—	18	—	20	19	23	—	25	—	25	27
M/640	—	0	—	—	—	—	—	26	—	22	—	25	—	25	—	27	—	28
M/1,280	0	—	—	—	—	—	25	—	22	—	25	25	26	—	29	—	27	27
M/2,560	—	0	—	—	—	—	—	—	—	26	—	27	—	23	—	25	—	26
M/5,120	1	—	—	0	—	—	26	—	29	—	28	23	26	—	21	—	25	27
M/10,240	—	11	14	17	10	15	—	18	—	22	—	22	—	22	—	22	—	23
Controls— nutrient medium alone	26	—	21	—	23	26	22	—	21	—	25	23	21	—	26	—	20	22
	—	28	—	22	—	27	—	25	—	23	—	20	—	21	—	22	—	23

* GSH = glutathione.

† Time: 1/5 = 1 hour, 5 minutes; etc.

medium in series C. Equivalent amounts of Tyrode were added to the serum contained in series A and B.

Trypanosomes, separated from blood corpuscles by centrifugation, were suspended in Tyrode, and 1 drop of this suspension was added to each tube of series A, B and C. The results are recorded in Table II.

Whereas a very small amount of glutathione, dissolved in a medium containing inactivated serum (Table II, series A) was lethal to trypanosomes, this lethal action was eliminated from concentrations below M/80, and greatly reduced in all, by the addition of liver catalase (series C). Substitution of unheated serum for heated serum also reduced the toxicity of glutathione (series B).

A reduction in the trypanocidal action of glutathione was also obtained by the addition of liver-extract to Tyrode. Further, a catalase preparation from rabbit blood was as effective as a liver preparation of the same catalase content.

No reduction in the trypanocidal action of glutathione was obtained by the addition of a preparation of horse-radish peroxidase to inactivated serum.

Trypanosomes themselves were found to possess a small but definite catalase activity, as determined by the liberation of oxygen from hydrogen peroxide in a Van Slyke volumetric apparatus, under suitably controlled conditions.

The trypanocidal activity of hydrogen peroxide was also examined. It was found that in 1 hour a concentration of hydrogen peroxide of 1 in $7\frac{1}{2}$ millions in Tyrode solution killed all the trypanosomes in a suspension containing approximately 1,200 organisms per cubic mm. In spite of their content of catalase, therefore, trypanosomes are killed by concentrations of hydrogen peroxide far below those which can be demonstrated by chemical means. This toxicity was reduced in the presence of fresh unheated serum, in agreement with the results of Reiner and Leonard (1932).

EXPERIMENTS WITH ASCORBIC ACID

The foregoing experiments support the hypothesis that hydrogen peroxide, produced during the autoxidation of glutathione, causes the trypanocidal action of this substance. Since ascorbic acid is another autoxidizable compound of biological importance, it was of interest to discover whether this substance gave results similar to those obtained with glutathione.

Different concentrations of ascorbic acid, neutralized with normal sodium hydroxide, were prepared in the usual way. These were made up in Tyrode diluted with inactivated serum (series A), or inactivated serum to which had been added either of two catalase preparations, (1) that used in the experiments with glutathione (series B), or (2) series C. The toxicity of the three series of ascorbic acid concentrations for a strain of *Trypanosoma rhodesiense* was then examined. The results are given in Table III.

TABLE III

Dilutions of ascorbic acid	Series A		Series B							Series C								
	Serum alone		Serum + preparation (1)							Serum + preparation (2)								
	0/30*	1/0	0/35	1/5	2/0	3/0	4/0	5/45	7/0	11/0	0/45	1/15	2/10	3/10	4/10	6/0	7/10	11/5
M/10	0	—	15	—	16	—	0	—	—	—	16	—	10	—	3	0	—	—
M/20	—	—	—	13	—	11	0	—	—	—	—	14	—	12	0	—	—	—
M/40	0	—	18	—	17	—	1	0	—	—	18	—	13	—	1	0	—	—
M/80	—	—	—	14	—	15	3	0	—	—	—	20	—	10	1	0	—	—
M/160	0	—	20	—	17	—	15	0	—	—	17	—	17	—	2	0	—	—
M/320	—	—	—	14	—	17	18	1	0	—	—	13	—	20	12	0	—	—
M/640	0	—	21	—	14	—	14	—	0	—	19	—	16	—	13	—	—	—
M/1,280	—	—	—	15	—	18	18	17	6	0	—	14	—	17	12	0	—	—
M/2,560	0	—	20	—	15	—	15	—	11	6	18	—	18	—	13	2	0	—
M/5,120	—	—	—	14	—	13	—	14	16	18	—	15	—	20	—	4	1	0
M/10,240	4	0	18	—	14	—	—	—	15	15	13	—	15	—	—	9	10	1
Control— nutrient medium alone	—	13	—	13	—	14	—	—	15	15	—	14	—	15	—	16	15	15

*Time: 0/30 = 30 minutes; 1/0 = 1 hour; etc.

It will be seen from Table III that ascorbic acid resembles glutathione in that very small amounts dissolved in Tyrode + heated sheep serum are trypanocidal, and that this trypanocidal action is considerably reduced by the addition of a liver catalase preparation. The death of the trypanosomes which occurred in series B and C after 3 to 4 hours' incubation will be discussed later.

It will be seen that the protective action of preparation (1) was greater than that of preparation (2). Further, the catalase activity of preparation (1) was found to be nearly three times that of preparation (2) when these activities were determined by the liberation of oxygen from hydrogen peroxide.

If the phenomena so far described are due to the production of hydrogen peroxide by autoxidation of glutathione and ascorbic acid, their demonstration should require the presence of molecular oxygen. In support of this, it was found that, in the absence of oxygen, the toxicity of ascorbic acid was strikingly decreased.

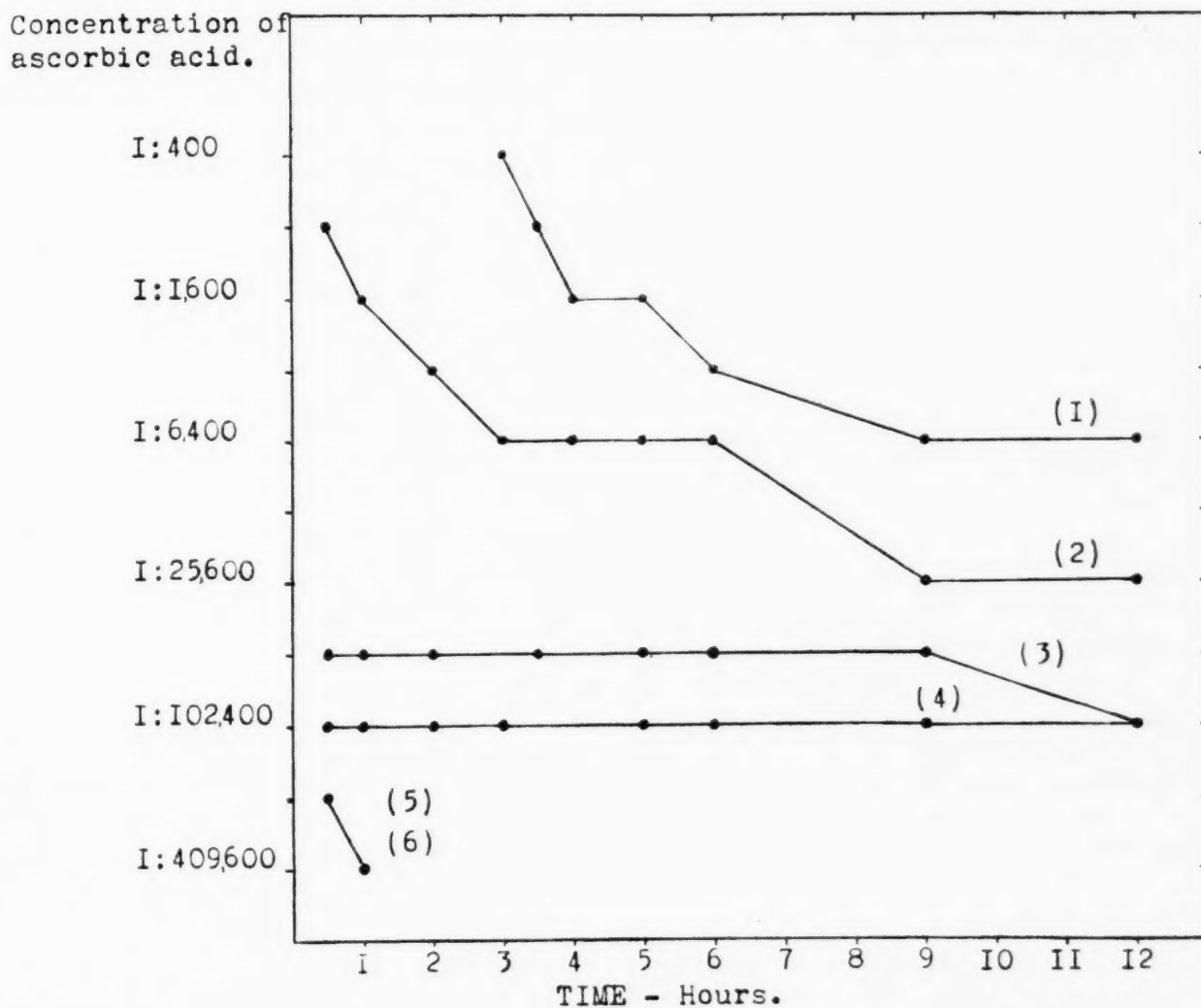
Ascorbic acid (0.46 mgm.), which, in the presence of air, killed all the trypanosomes in 2 c.cm. of a suspension within 30 to 45 minutes after mixing, had no lethal effect on the same volume of suspension *in vacuo* during a period of 10 hours. Thus, 5 evacuated tubes were prepared after thorough washing out with oxygen-free nitrogen, each containing approximately the same weight of solid ascorbic acid, 0.47 to 0.53 mgm., and 2 c.cm. of trypanosome suspension. After solution of the ascorbic acid in the trypanosome suspension, the tubes were incubated at 37° C. for different periods up to 10 hours. In each case all the trypanosomes were healthy when a tube was opened, and dead within 20 to 30 minutes after exposure to air.

It seemed desirable that a more active preparation of catalase should be used if possible. This was available through the kindness of Sir P. P. Laidlaw, who had prepared from horse liver a solution of catalase in alkaline sodium hydrogen phosphate by the method described by Keilin and Hartree (1936). This solution was not subjected to the final stages of purification, but was very much more active than any which had been used previously. With this sample of catalase all the previous phenomena were reproducible.

The evidence so far presented supports the conclusion that hydrogen peroxide is the agent responsible for the lethal action of solutions of glutathione and ascorbic acid; but the action of tissue extracts in inhibiting the oxidation of ascorbic acid (cf. Mawson, 1935) made it desirable to determine whether catalase exerted its protective action by inhibiting the oxidation of ascorbic acid, thus preventing the formation of some toxic oxidation product, rather than by destroying hydrogen peroxide as it was formed. The following two experiments, comparing the activity of the catalase preparation in reducing the trypanocidal effect of ascorbic acid, with its inhibitory action on the aerial oxidation of this compound, showed that this inhibitory action was not the factor concerned.

In the first experiment, the abilities of five different dilutions of the catalase preparation to reduce the trypanocidal action of ascorbic acid were

compared. Five series of tubes were prepared, each containing the same serial dilutions of ascorbic acid, in nutrient medium containing inactivated serum to which had been added different dilutions of the catalase preparation. A sixth series of ascorbic acid dilutions was prepared in medium to which no catalase had been added. Trypanosome suspension, prepared from a mouse infected with *T. rhodesiense*, was added to each tube, and the trypanocidal action of the ascorbic acid in each series was compared in the usual way. Curves have been plotted showing the concentration of ascorbic acid, in each series, in which all the trypanosomes in a suspension were killed after a given number of hours. These curves are reproduced in the figure. It can be seen that the protective



Trypanocidal action of ascorbic acid in nutrient medium containing:—

- (1) 1 part in 80 of catalase preparation.
- (2) 1 " " 800 " " "
- (3) 1 " " 8,000 " " "
- (4) 1 " " 80,000 " " "
- (5) 1 " " 800,000 " " "
- (6) No added catalase.

action of the catalase preparation was proportional to its concentration, a 1 in 80,000 dilution affording a slight but significant protection against the trypanocidal action of ascorbic acid, while 1 part in 800,000 gave none.

The second experiment showed that the addition of 1 part in 10 of the catalase preparation to inactivated serum did not increase the inhibitory action of the latter on the autoxidation of ascorbic acid.

Dilutions in phosphate buffer, pH 8.0 of (1) catalase, (2) inactivated serum, and (3) inactivated serum containing 1 part in 10 of the catalase preparation, were made in open test-tubes, each tube containing a volume of 4.0 c.cm. To each of these tubes, and to tubes containing buffer alone, 0.1 c.cm. of the neutral 0.5 per cent. solution of ascorbic acid, as used in the *in vitro* experiment, was added*. The tubes were incubated for 1 hour at 37° C. At the end of this time oxidation was stopped by the addition of an equal volume of 10 per cent. trichloroacetic acid, and the ascorbic acid remaining determined immediately by the method of Harris and Ray (1933). The results are recorded in Table IV.

TABLE IV

Dilutions of serum or catalase in buffer	Percentage oxidation of ascorbic acid after 1 hour at 37° C.		
	In buffer + catalase	In buffer + serum	In buffer + serum containing 1 part in 10 of catalase
1 : 1,000	24	0	0
1 : 5,000	24	0	0
1 : 25,000	24	36	43
1 : 125,000	43	43	43
1 : 625,000	43	—	43
1 : 3,125,000	43	43	43

Control : Ascorbic acid in buffer alone.

Percentage oxidation after 1 hour at 37° C. = 43.

It will be seen that the presence of 1 part in 10 of the catalase preparation in inactivated serum had no effect on the dilution of the latter which would inhibit the autoxidation of ascorbic acid, although, as shown previously, the presence

*A solution of ascorbic acid of this strength shows no measurable autoxidation over a number of hours.

of 1 part in 80,000 significantly reduced its trypanocidal activity. It would seem, therefore, that the action of the catalase preparation in reducing the toxicity of ascorbic acid for trypanosomes is not dependent on the power of this, as well as of other tissue extracts, to inhibit its aerial oxidation.

Evidence for the destruction of catalase by ascorbic acid. In all experiments in which the protective action of catalase preparations against the trypanocidal activity of ascorbic acid was examined, there was an initial protection against the toxic action of relatively high concentrations of the latter substance, which lasted for 1 to 4 hours. After this time, the trypanosomes remained healthy only in the presence of much smaller amounts of ascorbic acid, the exact concentration depending on the amount of catalase added. The phenomenon, to which reference has already been made, is apparent in Table III. A similar effect was never observed with glutathione, a concentration of catalase which was sufficient to remove the immediate trypanocidal action of this compound doing so throughout an experiment.

In connection with the above results, those of Euler and Larsson (1934) may be considered. These authors found that, while the activity of barley catalase was slightly increased in the presence of glutathione or cysteine hydrochloride, ascorbic acid had a destructive action on this as well as on horse liver catalase. It could, therefore, be argued that the phenomenon observed in the trypanosome experiments was due to the gradual destruction of catalase, over a number of hours, by the higher concentrations of ascorbic acid. Further work would be necessary to prove this, however, since Marks (1936) found that the rate of inactivation of preparations of mussel catalase was increased by the addition of glutathione, and that this compound also had a destructive action on stable preparations of beef-kidney catalase.

Chemical tests. No chemical evidence for the production of hydrogen peroxide during the oxidation of either glutathione or ascorbic acid has been obtained. Dr. H. King (personal communication) had previously been unable to demonstrate the formation of barium peroxide during the oxidation of glutathione in saturated baryta in the presence of oxygen, though this method had enabled him to demonstrate the formation of hydrogen peroxide during the aerial oxidation of leucomethylene blue. The same method applied to ascorbic acid gave an insoluble precipitate which, on analysis, was found to be a quantitative yield of barium oxalate.

Attempts to demonstrate the oxidation of nitrate in the presence of catalase or peroxidase, by ascorbic acid as well as by glutathione (Thurlow, 1925), were unsuccessful owing to the fact that, under the conditions of the experiments, no diazotization reaction could be brought about in the presence of either substance, and hence the Griess-Ilosvay test for the presence of nitrates was not effective. Nor was any evidence obtained for the formation of nitrate in solutions of sodium nitrite.

DISCUSSION

The above account presents a certain amount of evidence in support of the hypothesis that the formation of hydrogen peroxide during the oxidation of solutions of glutathione and ascorbic acid is responsible for their trypanocidal activity. The exact mechanism of this formation is, however, not apparent. It might be supposed (1) that the hydrogen peroxide is formed by aerial oxidation, either immediately or on standing, of solutions of the two compounds; or (2) that the oxidation takes place as a result of the metabolic processes of the trypanosomes themselves.

If the second alternative were the correct one, then it should be possible to demonstrate an increase in the rate of oxidation of either compound in the presence of trypanosomes. This possibility was examined in the case of ascorbic acid, but no evidence was obtained for an increase in the rate of its oxidation in the presence of trypanosomes.

The first alternative must now be considered, namely, that in spite of the lack of chemical demonstration, hydrogen peroxide is, indeed, formed during the autoxidation of ascorbic acid and glutathione. This formation might be immediate, or it might take place over a number of hours. With ascorbic acid, evidence that the formation of hydrogen peroxide was immediate was given by the fact that the trypanocidal activity was not increased by 6 hours' incubation at 37° C. It was found, also, that a neutral or slightly alkaline solution of ascorbic acid, prepared in the presence of air, was as toxic for trypanosomes when added to a suspension of these *in vacuo* as it was when added aerobically. If, on the other hand, as mentioned previously (p. 410), amounts of solid ascorbic acid, small enough to have no appreciable effect on the pH of the medium, were added to a suspension of trypanosomes *in vacuo*, the organisms remained healthy over a number of hours, although they were killed within 20 to 30 minutes after exposure to air.

The behaviour of serial dilutions of glutathione is noteworthy. It was found that strong solutions showed only a delayed trypanocidal action, while more dilute solutions had an immediate toxic effect. It is well known that reduced glutathione is readily oxidized by hydrogen peroxide in the presence of catalyzing agents such as copper, so that it might be argued that in the stronger, less toxic concentrations of glutathione the traces of hydrogen peroxide formed are rapidly destroyed by the large excess of the former substance. As the concentration of glutathione was decreased, in serial dilution, so the destructive action on any hydrogen peroxide present would be decreased, and its trypanocidal action, therefore, would become apparent. As dilution was continued, a lower non-toxic limit would, of course, be reached. In support of this hypothesis is the fact that the toxicity of a solution of ascorbic acid was much reduced in the presence of these non-toxic concentrations of glutathione. The point has not, however, been further investigated.

To summarize, therefore, it can be stated that, in neutral or slightly alkaline solutions of glutathione and ascorbic acid exposed to air, a substance is rapidly formed which is very toxic for trypanosomes *in vitro*. Evidence has been produced for the identity of this substance with hydrogen peroxide.

It is tempting to suggest that the destructive action of ascorbic acid and glutathione which has been observed in other connections—for example, vaccinia virus (Kligler and Bernkopf, 1937) and tubercle bacillus (Boissevain and Spillane, 1937)—may, in fact, be due to a similar production of hydrogen peroxide.

ADDENDUM

In a previous communication (Strangeways, 1937) it was recorded that relatively concentrated solutions of glutathione inhibit the trypanocidal action of arsenoxides by the formation of non-toxic thioarsinite, but that dilution of such a mixture, reducing proportionately the concentrations of glutathione and arsenoxide alike, causes the toxicity of the latter to become again effective. It was then suggested that this effect was due to hydrolysis of any thioarsinite in the presence of preponderating water molecules. In the light of the observations now recorded, these earlier ones obviously need to be reconsidered, with a view to another possibility. These earlier experiments were carried out in a medium containing fresh unheated serum, with a thick suspension of trypanosomes, so that abundant catalase was originally present. This, however, might quite conceivably be put out of action by the arsenoxide. If this were the case, then the zone of trypanocidal activity might be due, not to the liberation of arsenoxide by hydrolysis, but to the presence of hydrogen peroxide in the dilutions of glutathione.

This possibility, of accounting for the dilution effect on mixtures of glutathione and arsenoxide, is shown to be inadequate, however, by the fact that the lethal action of the diluted mixture is much more effective on normal trypanosomes than on those of an arsenic-resistant strain; while the lethal action of glutathione alone, due to the formation of hydrogen peroxide, is equally effective on normal and arsenic-resistant strains of trypanosomes.

ACKNOWLEDGEMENTS. It is a pleasure to record my indebtedness to Dr. H. King, F.R.S., for his continued advice, and to Sir Patrick Laidlaw, F.R.S., and Dr. F. G. Young, for their help during the course of this investigation.

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SOME OBSERVATIONS ON MOSQUITO LARVAE DYING IN ANTI-MALARIAL OILS AND OTHER SUBSTANCES

BY

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(Received for publication 21 August, 1937)

INTRODUCTION

My excuse for publishing these preliminary notes is that they proved of some interest to a few who knew of them. Dr. Coggeshall, of the Rockefeller Medical Institute, New York, has specially encouraged me to do so, as he wished to communicate my observations to a colleague who is working on a related subject.

In many, if not in all, descriptions of work on mosquito larvicides, only very brief mention is made of the signs that were considered sufficient for a larva to be called 'dead.' Sometimes their failure to wriggle if touched (Hacker, 1925, p. 3; Corbett and Hodgkin, 1931, p. 3), or their inability to reach and remain at the air surface (Green, 1924; Ramsay and Carpenter, 1932), or simply the fact that they were motionless and upside down (Ginsburg, 1929), was taken as evidence that they were dead. A chance observation made while I was working on material from the Department of Entomology in the London School of Hygiene and Tropical Medicine, during the course for the D.T.M. and H., showed that the heart muscle of mosquito larvae is sometimes very quickly stopped by substances that gain access to their breathing-tubes. In the Ross Institute of Tropical Hygiene further opportunities of study arose, and some observations made there are now published, in the hope that, when in future work is done on anti-malarial larvicides, they may be of some value.

I wish to express my thanks to Sir Malcolm Watson, Director of the Ross Institute, for the opportunity of doing this work and for his permission to quote from a report which is still in the press; and to Dr. B. B. Wigglesworth, for helpful elucidation on points of the larval anatomy.

I should also like gratefully to put on record the co-operation of the Asiatic Petroleum Company, through Mr. Braybrook and others, in every phase of the work and by supplying samples of the following oils, often at short notice:

Barnes Cross kerosene.	Roumanian + 10 pourpoint.
Miri Cross kerosene.	Roumanian kerosene.
Barnes solar oil.	Roumanian fuel oil.
Tarakan fuel oil.	Roumanian gas oil.
	Medicinal liquid paraffin.

In some experiments oil of citronella was used in 1:12 dilution with medicinal liquid paraffin.

ANATOMY

Only brief mention need be made of the arrangement of the heart and breathing-tubes in mosquito larvae, for very complete observations have already been published on this subject by others (Nuttall and Shipley, 1901 ; Howard, Dyar and Knab, 1912, pp. 84-98).

If we look at a larva under a lens, two dark lines, the tracheae, will be seen running the whole length of the abdomen, and waving inwards and outwards with a regular rhythm. The movement is due to the heart muscle, which lies between the two tracheae and seems to be attached to them so that they move as the heart pulsates. The pulse goes from the tail forward. The first movement seems to be a contraction of the longitudinal muscles, which in places throws the tracheae rapidly outwards. Then, at intervals, some transverse heart muscle bundles, one in each body segment, contract, and in so doing pull the tracheae at these points quickly together again. Between these points the tracheae appear just to swing together more slowly. The tracheal movement is entirely dependent on the heart pulsation and is, therefore, a very obvious indicator of the heart's activity.

THE METHOD

The larvae used were third and fourth instars of *Anopheles maculipennis* and *Culex fatigans*. As controls younger larvae were used, in order that they could easily be distinguished. The larvae were observed during the experiments under the 2/3 power of a microscope or under a low-power dissecting microscope. They were in dishes made of optical glass blocks 2 in. square and 1/3 in. deep, with a hollow ground concavity of 1/2 in. diameter on the upper surface. The dishes held conveniently about 2 c.cm. of water. The water used for storing larvae both before and after the experiments was rain-water. Individual larvae were kept in small glass bottles, 2 in. by 1 in., about half filled with water, and open to the air, though lightly corked. Larvae before use were stored together in a much larger jar, covered by netting. Their food was biscuit-meal. Sand was added for the anopheline larvae. For those larvae which were kept individually, fresh water was daily added to their bottles, but the old water remained as well. Each larva was examined microscopically before being used, to see that the heart was clearly visible and the rhythm regular. This was important, especially in the more darkly pigmented anophelines.

One drop of an oil was delivered from a fine pipette on to the edge of the water in the dish after each larva had been thus examined. The nature of the spreading film was noted. Time was recorded by a large and convenient stop-watch, and notes were made every few minutes of the behaviour of the larva. Especial watch was kept for the moment when oil began to run up the syphon of culicines or the tracheae of anophelines, and the time when this occurred

was recorded. If the oil was acting very quickly, notes were dictated to an assistant.

In all cases an observation was made of the time taken for the heart to stop, when the larva remained until the end under the film of oil.

In later experiments, some larvae were removed from the oiled water as soon as it was seen that the oil had begun to enter the breathing-tubes. They were transferred by means of a camel-hair brush. They were washed once or twice in changes of tap-water or rain-water, and usually very little or no film appeared on the rain-water into which they were finally put. Before being put individually into storage bottles they were each examined microscopically, and the degree to which oil then filled the breathing-tubes was noted. The behaviour of the heart muscle was also observed, and times were recorded.

A larva surviving after an experiment was examined at least twice daily, and no larva was thrown away until it had been examined microscopically, even though it was obviously 'dead.'

Paris green was added pure to the dishes, a very little being blown from a dry pipette on to the surface of the water. Watch was kept under the microscope for the moment when particles of the poison were seen to pass through the neck from the head into the thorax, after feeding and swallowing activities by the larva.

Mechanical asphyxiation was carried out by filling a lower dish with water into which the larva was placed, and by sliding over it an upper dish, taking care that no bubble of air was enclosed below. Through the two dishes together constant watch under the microscope could be kept upon the behaviour of the larva.

SOME OBSERVATIONS

Oils

After a drop of oil is placed on water in which a larva is swimming, the time taken for a particular type of oil to penetrate the tracheal openings may vary from only a few seconds to 10 minutes or even longer.

A great deal of work has been done on this aspect of anti-malarial oils, and much is known about the physical conditions preceding the entry of oil (Hacker, 1925, pp. 11-62; Wigglesworth, 1930; Corbett and Hodgkin, 1931, pp. 8-10). However, it may be of value to record one or two factors that seem, in these experiments, to have acted physiologically in bringing about a quick entry of the oil.

In contact with some quite good spreading oils (Tarakan; Roumanian + 10 pourpoint) the larva may lie quietly and seems to be in no way stimulated by the oil touching its skin. But in others, the more toxic oils, the larva begins to wriggle vigorously as soon as it hits the oil film. It seems as if the skin of the larva was irritated by some substance in the oil. During the movements the larva comes more intimately into contact with the oil. In any oil, but

especially in these very 'irritating' oils, the larva is seen very soon to bend round on itself and clean its tracheal openings with its mouth parts. And it is precisely this performance which so frequently can be seen to precede the entry of oil into the tracheae. It seems as if the larva, by its attempts at cleaning itself, smears the tracheal openings and brings the oil into a more intimate contact with the tracheal lining. Then the oil spreads in along the tubes.

For the first few minutes after the oil enters the tracheae the larva is usually seen to be restless and active in its movements. These bending movements seem to aid the spread of the oil along the tubes towards the thorax, for between bouts of activity the oil meniscus is usually seen to remain stationary. It moves forward when wriggling begins again.

The entry of oil into the tracheal system is easily seen in culicine and anopheline larvae. And the moment when the heart muscle ceases to contract can be accurately noted. The heart muscle rhythm is almost always irregular for some time before the heart finally stops.

Some oils take very much longer to stop the heart than do others, after each has been seen to enter the tracheae. These may be called the less toxic oils. The heart may still be beating more than 6 hours after the oil has entered the tracheae.

If the action is rapid, the stages in the heart's derangement are not so easily defined, but the moment when pulsation stops is clearly seen. This may occur in less than 2 minutes from the moment when oil first enters the tracheae.

When a toxic oil enters the tracheae the heart-beat ceases in the tail end before it does in the thorax or front end of the abdomen. It would seem possible, therefore, that the toxic portion of the oil penetrates the lining of the trachea and stops the heart locally.

In both culicine and anopheline larvae the pulsation rate of the heart, normally about 50-70 resting, rising to 90-100 after prolonged wriggling, usually gets slower before the larva dies. Then with some oils the regular rhythm is broken, so that, say, 1 in every 2 beats is dropped, as if the muscle were unable to contract. With other oils it has been noted that the pulsation rate, after being slowed, begins to increase over the last 20-30 minutes before the heart finally stops. In one larva the rate at the sixth abdominal segment rose from 29 to 59 per minute during 26 minutes of observation, and in this larva the last beat was actually watched. The pulsation rate rose, but the strength of the contractions seemed clearly to fail.

As a general rule, in these experiments the heart has survived longer in those larvae which were washed soon after the oil was seen entering the tracheae than in the larvae which were left under the oil film.

The toxicity of a mixture of highly toxic and non-toxic oils seems to bear a relationship to the degree of dilution of the toxic oil; the non-toxic oil seems to act merely as a vehicle. For instance, a kerosene which stopped the heart of an anopheline in 2 minutes was added to Tarakan fuel oil which stopped

the heart only after more than 4 hours, in such proportions that the mixture of the two just began to spread well (1 in 7). One drop of this mixture was then put on water in a dish containing an anopheline larva. After the mixture entered the tracheae, the time taken to stop the heart was 9 minutes. That is to say, in diluting a toxic oil 7 times, we weakened its killing power only 5 times, whereas this seventh part of a kerosene strengthened the killing power of the less toxic Tarakan 25 times.

If a strongly toxic oil comes into close contact with other parts of a larva than the tracheal system, the muscles of those regions are usually thrown into convulsions; this sometimes precedes the entry of oil into the tracheae.

If a larva comes into contact with a film of less toxic oil, and this oil fills the tracheal system, and if then the larva comes into contact with a film of highly toxic oil, the latter oil will take much longer than usual to stop the heart, and any part of the heart muscle may stop first. It would seem in such a case that the toxic portion of the oil must penetrate the covering of the larva, possibly through the thinner parts about the neck and between the segments, or through the lining of the gut, and that these routes to the heart muscle are slower than through the tracheal lining.

If a larva comes into contact with some types of heavy non-toxic oil—such as liquid medicinal paraffin or Roumanian fuel oil—so that oil droplets adhere to hairs ventrally or about its head, or to the anal gills, the larva may be held fast in a position in which its tracheal openings are not in contact with air. One culicine larva remained thus overnight, and during the next morning was able to free itself. It was still alive on the second day. Oil had penetrated only the left trachea. Anopheline larvae have so far not survived this treatment.

A larva whose tracheal system is quite filled with a heavy non-toxic oil seems to be too heavy for its wriggling to carry it far upwards. If the water is deeper than about 2 in. the larva may often fail to reach the air surface. This is possibly because oil, which is relatively heavy, has replaced air, which is light, inside the larval body. At the same time, the muscles of the larva, being partly asphyxiated, are more rapidly fatigued.

No larva in which the heart has been quite stopped by a toxic oil has so far been seen to show any signs of recovering. But various degrees of recovery have been seen in other experiments if the larvae have been carefully 'nursed.' For instance, the heart-beat after becoming very slow and weak may return to a normal rhythm, and spontaneous wriggling may return in a larva which had ceased to respond to touching.

Three culicine larvae were placed under a film of non-toxic oil (Tarakan) and watched until it was seen that oil had just penetrated the syphon; each was then removed into fresh tap-water and washed; two of these subsequently pupated and hatched into adults, and one began pupation but failed to complete it.

One culicine larva was observed beneath a film of non-toxic oil (Tarakan) for 20 minutes, by which time the left trachea was quite full of oil and the right

one full to the fourth abdominal segment. It was then taken out and washed. The heart continued to beat. On the third day thereafter the heart was still beating, but the left trachea was collapsed at the inter-segmental joints, while the right trachea was filled again with air. On the fourth day the larva was dead. A control larva in the same dish pupated normally.

But recovery has not yet been seen in any larva which had got even a minimal amount of a highly toxic oil into the end of one trachea. This is possibly because the heart is stopped in less time than the larva takes to get rid of the particular oil from its trachea.

The variation in time taken to stop the heart, after any particular toxic oil has entered the tracheae, was in the region of ± 20 per cent. in these experiments.

It seems almost certain that some oils are eaten by larvae, and Tarakan oil has been seen in the gut of larvae before and after death. Movements of the gut are not infrequently seen after the heart has ceased pulsation.

To sum up these observations : while a larva is dying after contact with an oil film, convulsions are frequently seen quite early. When the tracheal system is full of oil, the larva usually sinks and lies at the bottom, either motionless or making only inefficient efforts to reach the air surface. After a while, the larva ceases to react to touching, nor does it wriggle if drawn up with some water into a pipette ; but even in this state pulsation of the heart may still be seen. The heart may not cease for some hours after the larva is insensitive to touch. If the heart is stopped quickly, convulsive movements may continue for a few minutes longer ; the muscle of the gut is usually the last to die. Some degree of recovery may be expected if the less toxic oils are used and if the tracheal system is only incompletely filled by the oil.

Mechanical Asphyxiation

If a larva is prevented mechanically from reaching the air surface for long enough, it will die ; but the time taken to do so varies according to the amount of oxygen available for its use in the water (Howard, Dyar and Knab, 1912, p. 89 ; Ramsay and Carpenter, 1932).

During this process of asphyxiation, changes in the rhythm and frequency of the heart's pulsation may be observed. One anopheline larva in tap-water was prevented from reaching the surface mechanically for 2 hours, 20 minutes. At the end of this time the heart pulsation had slowed from 120 beats per minute to 30 per minute. Its rhythm was irregular, and sometimes there were pauses of as long as 20 seconds before spontaneous pulsation began again. The other muscles of the body and of the head and mouth parts were convulsing irregularly and often. The whole head at times vibrated gently on the thorax. At this point the water in which the larva lay was aerated again, but the larva did not reach the air surface by its own movements in 10 minutes, nor was there any reaction to touch. The larva was then carefully brought to

the surface, so that it *adhered* and its breathing-tubes could open again. During the next 40 minutes the heart was carefully watched, the rhythm became regular again, but there was still no definite reaction to touch. It was left at the surface overnight. During the following day the larva could feed actively with complete rotation of the head, and the heart pulsation was strong and regular. It reacted quickly to shaking or touching. On the second morning it was, however, found dead.

A larva in a state of incomplete asphyxiation seems unable to reach the air surface if it has far to climb. In very shallow water it may do so almost by accident, and it may then recover; but, if it does not reach the air surface, the process of suffocation will continue until it becomes complete and the larva dies.

Paris Green

About 10-20 minutes after a larva, either culicine or anopheline, has been seen to swallow a particle of Paris green, heaving convulsions of the thorax and body have always begun. Movements of the feeding brushes may continue after convulsions have started. Defaecation is more frequent and the whole gut may be emptied of food. Pulsation of the heart may often be seen after the larva has become insensitive to touch, and may continue for as long as 4 hours after the Paris green was seen being swallowed. But even of those larvae which were put into clean water immediately after swallowing some Paris green none showed any signs of recovering.

Certain proprietary preparations of colloidal Paris green were tested, and preliminary observations suggest that they are not inferior to the solid preparations in killing larvae.

Naphthalene

Bacot (1916) has reported that naphthalene flakes on water are, with few exceptions, fatal to all larvae for 48 hours. A few experiments have been done with naphthalene crystals floating on water, in which it is said to be insoluble, and with the crystals dissolved in oil. But the results are inconclusive.

In two culicine larvae exposed to naphthalene crystals on the water of the dish, a slow irregular rhythm of the heart-beat developed. The first larva was exposed for over 2 hours and the second for over 1 hour. The larvae were returned to fresh water, and in both the heart rhythm returned to normal. The first lived two days and the second five days. The second larva was very sluggish on the fourth and fifth days, and its heart rhythm was slow ($P = 45$ per min.) before finally ceasing.

An oil in which excess of naphthalene crystals were allowed to dissolve stopped the heart no more quickly than the pure oil alone in 3 anopheline larvae (about 70 min.). In a fourth anopheline the heart was stopped very much sooner (10 min.).

THE FUTURE

' Up to 1914 no one thought that it was possible to use oil as an anti-malarial method on running water. Everyone believed that for an oil to kill larvae a permanent film was necessary. On running water the oil film is rapidly broken up and carried away down the stream. Permanency is, therefore, a physical impossibility, but happily of little or no importance in most malarial countries. And on ponds winds often blow the oil to one side and so prevent there being a permanent unbroken film. The discovery by the Director in 1914 of a mixture of oils, which killed all anopheles larvae in a fast-running stream, which killed the grass on the side of the stream, and which produced a profound change in the algae of the stream, showed the prime importance of toxicity in an anti-malarial oil ' (Department Report of the London School of Hygiene and Tropical Medicine, 1937).

Ginsburg (1929) and others (Hacker, 1925, p. 9) considered that nerve tissue was the site of this toxic action. But, beyond this, few experiments seem to have been reported of attempts to define more clearly the pharmacology of these oils. From the present work it would appear probable that among other tissues the heart muscle of the larva is rapidly affected by the more toxic oils.

Paris green, on the other hand, does not seem to act on the heart muscle primarily, but behaves in larvae like the gastro-intestinal poison which it is in man.

It would appear from these experiments that the dose of oil which a larva receives is one of the factors that determine how long it will survive after contact with any particular film. This may be an important consideration when oiling a stream, if the oil used is not very toxic. And further work may have to be done to find out how much oil does in fact penetrate the breathing-tubes of stream-breeding larvae under various conditions in the field.

If the dose of oil is small and the oil itself not toxic, it would appear that some larvae may recover, even sufficiently to pupate and hatch. It may be of importance to determine this chance of recovery before a particular oil is recommended for use in the field.

If these observations are confirmed, the power of an oil to stop the contractions of the heart muscle may prove of value when the toxicity of an anti-malarial oil is being studied. It may be desirable in future to investigate the comparative rapidity with which different oils and different substances in these oils stop the heart muscle, and to use this knowledge when planning the composition of an anti-malarial mixture. This method has, in fact, just been used by the staff of the Ross Institute in planning what mixtures of local oils would be effective in a part of the world where new anti-malarial work is shortly to begin.

Further work is needed on the property of ' stickiness ' in an anti-malarial oil, that is, its power of adhering in droplets to the exterior of larvae and to grasses, stones and other things at the surface of water. It is a property which

might perhaps enhance the value of a toxic oil that is sprayed along the edges of a stream. It might keep more oil for a longer time around the vegetation at the sides, where stream-breeding larvae are as a rule to be found; and it might help to spoil these regions as a place for adult mosquitoes to lay their eggs. Since an anti-malarial oil is usually required to burn the vegetation on the banks, such 'stickiness' might retain the oil in the places where this last effect will most readily be brought about.

It will probably always be more economical to use only one type of anti-malarial oil, both for running water and for pools. Any oil which will control the stream-breeding larvae will also be effective in standing water, so it would appear that work upon the production of permanent films of oil should be preceded by work upon the oil's toxicity.

CONCLUSION

Much is already known about the killing properties of different petroleum distillates. But methods so far described in measuring this power seem to be open to two criticisms. First, the measurement of the killing time has begun at the moment when oil was placed on the water in which the larvae were, instead of from the moment that oil entered the breathing-tubes. These two moments may be several minutes apart. Second, the decision that a larva was dead has rested usually upon the absence of any reaction to touch or the inability of the larva to rise to the air surface. It would seem that under certain conditions at least larvae are capable of reviving, even though they have reached either of these states.

It is suggested that a more accurate measurement of the killing power may be obtained in future by measuring the interval which elapses between the entry of oil into the breathing-tubes and the moment when the heart muscle finally ceases to contract.

One thing which has long been sought in an anti-malarial oil is reliability of composition (Watson, 1921; Barrowman, 1931). It seems possible that by working with one property alone, such as the oil's power to stop the pulsation of the heart, we may sooner learn how to create and maintain a dependable mixture of oils than if we continue to use the less exact methods of measuring killing time employed so far by many workers.

What we want an anti-malarial oil to do should be clearly defined, and we should have as simple and as rapid a method as possible of measuring the power of the oil sample to do what we want of it. In this way we should be able to compound an oil mixture in which each fraction was included for a specific purpose: one fraction because it was toxic up to a known dilution; another because it burnt vegetation; another because it was dark and heavy and 'sticky'; the whole planned so that the initial spread was wide. If such a mixture were also a cheap one, it would have all the properties which General

Gorgas (Howard, Dyar and Knab, 1912, p. 9) required of the perfect anti-malarial oil, a matter of importance in tropical hygiene.

SUMMARY

1. Some observations are recorded on the behaviour of culicine and anopheline larvae which were put into contact with oils, Paris green and solutions of naphthalene, and on others which were partly or completely asphyxiated mechanically.

2. It is recorded that the heart muscle of larvae is disturbed in its rhythm, and sometimes rapidly stopped, when certain oils enter their breathing-tubes, and that other oils do not produce these effects.

3. It is suggested that in future work on anti-malarial oils this observation may be made the basis of comparative measurements of the oils' toxicity.

4. The possibility of a larva recovering sufficiently to pupate and hatch under certain circumstances is discussed.

5. Certain other properties of a good anti-malarial oil are discussed. These are its 'stickiness,' its power to burn vegetation, its killing power in running water, its cost and its reliability.

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PARASITIC INFECTIONS IN A SWAN AND IN A BROWN TROUT

BY

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(Received for publication 7 September, 1937)

I. SWAN

Early in July, 1937, one of the swans living on the lake in Sefton Park, Liverpool, was found to be ill and was removed to another area. Shortly afterwards it died. The bird was sent to the Pathological Department of the Veterinary School, University of Liverpool, for examination, and parasites in large numbers were found in its intestine. The bird was extremely emaciated, the intestine was very inflamed, and the cause of death was undoubtedly due to the heavy parasitic infection, no less than three different species being found.

1. CLASS CESTODA

FAMILY HYMENOLEPIDIDAE RAILLIET AND HENRY, 1909

SUBFAMILY HYMENOLEPIDINAE RANSOM, 1909

Genus *Hymenolepis* Weinland, 1858

Hymenolepis (*Weinlandia*) *gracilis* (Zeder, 1803)

This tapeworm has not previously been recorded from the swan. Normally it occurs in ducks and geese. The parasite measures from 12 to 27 cm. in length and from 1 to 2 mm. in breadth. The rostellum is usually elongated and bears 8 hooks, each measuring about 78μ in length and having a characteristic shape.

The eggs, when passed in the faeces of the infected duck or goose, are swallowed by various species of *Copepoda*, such as *Cypria ophthalmica*, *C. viridis*, *Diaptomus* spp. and others. In these hosts the hexacanth embryo escapes from the egg, bores through the intestine of the crustacean and becomes a cysticercoid in the body cavity. The infected crustaceans are then swallowed by ducks and geese, and the cysticercoids become adult in the intestine of these hosts.

We obtained some hundreds of specimens of this parasite from the intestine of the swan (*Cygnus olor*). The number, size and shape of the hooks corresponded exactly with those of *H. gracilis*, and so also did the disposition of the testes. The worms, however, measured only 2 cm. in length and approximately 1.5 mm. in breadth. The posterior segments were just mature, none being gravid. We are satisfied that they were all young worms which would have attained their normal size in due course, had the bird lived.

Although this parasite has apparently not been recorded previously from the swan, its occurrence in this host is not remarkable; thus *Hymenolepis setigera* (Frölich, 1789), *H. lanceolata* (Bloch, 1782), and others are found both in geese and in swans, and there seems no valid reason why *H. gracilis* should not also be common to both these birds.

The following is a list of tapeworms which have been recorded from the swan (*Cygnus olor* and *Cygnus cygnus*), together with a table showing the number and size of the hooks.

			No. of hooks on head	Size of hooks
<i>Drepanidotaenia lanceolata</i> (Bloch, 1782)	8	31 to 35 μ
<i>Hymenolepis setigera</i> (Frölich, 1789)	10	25 to 44 μ
<i>Hymenolepis gracilis</i> (Zeder, 1803)	8	76 to 82 μ
<i>Hymenolepis aequabilis</i> (Rudolphi, 1810)	10	27 to 34 μ
<i>Hymenolepis megalops</i> (Nitzsch, 1829)	16-23	14 to 18 μ
(According to Lühe and to Fuhrmann, the rostellum is rudimentary and unarmed, but Neveu-Lemaire states that it is armed as above.)				
<i>Hymenolepis micrancristota</i> (Wedl, 1855)	20	10 μ
<i>Hymenolepis anatina</i> (Krabbe, 1869)	10	65 to 72 μ
<i>Hymenolepis creplini</i> (Krabbe, 1869)	10	21 to 24 μ
<i>Hymenolepis liophallos</i> (Krabbe, 1869)	10	35 to 38 μ
<i>Hymenolepis fona</i> (Meggitt, 1933)	10	70 to 74 μ
<i>Nematoparataenia southwelli</i> (Fuhrmann, 1923)	1,000	6 μ

2. CLASS TREMATODA

SUPERFAMILY **Fascioloidea** STILES AND GOLDBERGER, 1910

FAMILY PSILOSTOMIDAE ODHNER, 1913

SUBFAMILY PSILOSTOMINAE LÜHE, 1909

Genus *Psilostomum* Looss, 1899

Psilostomum cygnei sp.nov.

Hundreds of these trematodes were obtained from the small intestine of the swan. They were white in colour, and the size varied from 1 mm. to 2.5 mm. All are characteristically Y-shaped, the limbs of the Y being formed by the oral and ventral suckers. The body is slender, elongated, and tapers to a bluntly rounded posterior extremity.

The oral sucker leads directly into a muscular pharynx, which is slightly larger than the sucker itself. A very short oesophagus is present; the limbs of the caeca are simple and pursue a somewhat sinuous course, to terminate just short of the posterior extremity. The ventral sucker is very striking. It projects, almost at right-angles, from the anterior extremity, at about the level of the posterior border of the pharynx. In almost every instance it measured twice the size of the oral sucker.

The testes are large, round, placed one behind the other, and situated in the posterior half of the parasite. The cirrus sac is large and elongated, but the musculature is poorly developed. The genital pore opens between the oral and ventral suckers, just to the left of the mid line.

The ovary is round, almost the size of the testis, and is situated anterior to the anterior testis, to the left of the mid line. The uterus is short and is

confined to the space between the ovary and the pharynx. In gravid specimens it usually contained about three eggs, at the most six. These are operculated, yellowish-brown in colour, and measure about 72μ in length by 55μ in breadth. The vitelline glands are well developed and extend from the level of the ovary almost to the posterior extremity. They lie lateral to the ovary and testes, but behind the posterior testis they coalesce across the mid line.

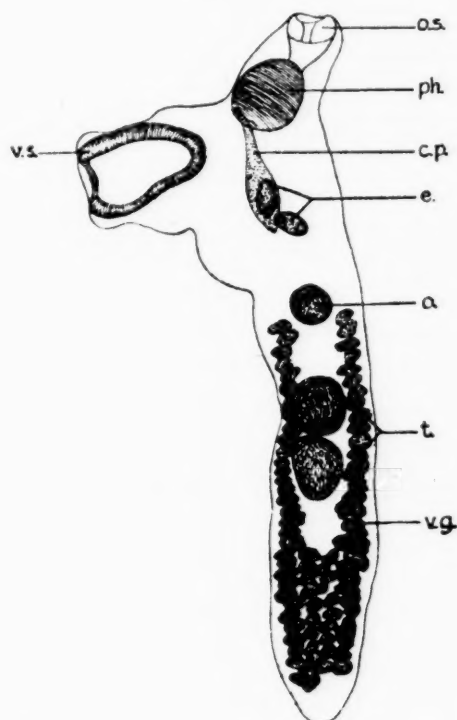


FIG. 1. *Psilostomum cygneï* sp. nov.
Lateral view. ($\times 40$)



FIG. 2. *Psilostomum cygneï* sp. nov.
Ventral view. ($\times 40$)

EXPLANATION OF LETTERING

c.p., cirrus pouch.
e., eggs.
e.v., excretory vessel.
g.c., gut caecum.
g.p., genital pore.

m., mouth.
o., ovary.
o.s., oral sucker.
o.t., organs of attachment.
p.h., pharynx.

t., testes.
v.d., vas deferens.
v.g., vitelline glands.
v.s., ventral sucker.

Odhner (1913) erected the family *Psilostomidae* to accommodate the following 5 genera :

- Psilostomum* Looss, 1899.
- Psilochasmus* Lühe, 1909.
- Psilotrema* Odhner, 1913.
- Apopharynx* Lühe, 1909.
- Sphaeridiotrema* Odhner, 1913.

The worms in the family *Psilostomidae* are parasitic in the intestine of aquatic birds, chiefly ducks and water-hens, though some species have been recorded from water-rats and also from fowls. Up to the present they have

not been recorded from swans. The family includes those trematodes in which the ovary is situated anterior to the testes; the cirrus pouch is very elongated, only slightly muscular, and the vesicula seminalis is small or absent; the suckers are large; the vitelline glands are well developed and situated laterally, except posterior to the testes, where they join across the mid line.

The two subfamilies *Psilostominae* Lühe, 1909, and *Orchipedinae* Skrjabin, 1913, are separated on account of the fact that in the latter each parasite possesses more than two testes.

3. CLASS **ACANTHOCEPHALA** RUDOLPHI, 1808
 FAMILY CORYNOSOMIDAE SOUTHWELL AND MACFIE, 1925
 Genus *Filicollis* Lühe, 1911

Filicollis anatis (Schränk, 1788) Lühe, 1911

Several males and females belonging to this species were found in the intestine. As is usual, the heads of the males were attached to the mucous membrane of the intestine, whilst those of the females had penetrated the intestinal wall and were lying on the peritoneal surface. Although the parasites were morphologically indistinguishable from *Filicollis anatis*, the uterine eggs were enormous and measured from 195 to 325 μ , whereas the usual size of *F. anatis* eggs is from 56 to 70 μ . We regard this as an abnormality.

II. BROWN TROUT

1. SUPERFAMILY **Spiruroidea** RAILLIET AND HENRY, 1915
 FAMILY THELAZIIDAE RAILLIET, 1916
 Genus *Cystidicola* Fischer, 1798

Cystidicola farionis Fischer, 1798

Enormous numbers of this parasite were found in the swim-bladder of the trout.

Shipley (1908) recorded similar parasites from trout dying in a stream in Herefordshire. The usual number of parasites in each fish was from 8 to 18, and he doubted whether these parasites were the cause of death, as they are usually regarded as harmless. In the case of our specimen, we consider that, apart from the heavy infection of the gills of the trout with other parasites (*vide infra*), the heavy infection with *Cystidicola farionis* was in itself quite sufficient to cause death.

2. CLASS **TREMATODA**
 ORDER **MONOGENEA** VAN BENEDEN
 FAMILY MAZOCRIIDAE NOV.
 Synonyms: OCTOCOTYLIDAE VAN BENEDEN AND HESSE
 OCTOBOTHRIIDAE TASCH., 1879

Genus *Mazocraes* Hermann, 1782

Synonyms : *Octobothrium* Leuckart, 1827

Octostoma Kuhn, 1829

Discocotyle Diesing, 1850

Diclidophora Diesing, 1850

Octocotyle Diesing, 1850

Octoplectanum Diesing, 1858

Baylis and Jones (1933) state that the name *Octobothrium* must fall into synonymy of *Mazocraes*, as *Octobothrium lanceolatum*, the genotype of *Octobothrium*, is identical with *Mazocraes alosae*.

Mazocraes sagittatum

Synonyms : *Octobothrium sagittatum* Leuckart, 1842

Discocotyle sagittatum (Leuckart, 1842) Diesing, 1850

Over a hundred specimens of this ectoparasitic trematode were obtained from the gills of a brown trout found dead in a reservoir in North Wales. The gills were very pale—almost white—and were covered with a thick mucus. The association of pallid gills, covered with mucus, and the large number of parasites present strongly suggest that the immediate cause of death was due to these worms.

MacCallum (1917) recorded a closely related species of the genus *Diclidophora* from the gills of certain marine fishes, and stated that 'it is not found in any great numbers anywhere, in fact is always found singly, and consequently cannot be said to be a menace to the welfare of its host in any case.'

The species *Mazocraes sagittatum* is not only widely distributed, but occurs on the gills of fresh-water, estuarine and marine fishes. We are of opinion that the three species recorded by MacCallum (1917), under the names *Diclidophora merlangi* from *Merluccius bilinearis*, *D. prionoti* from *Prionotus carolinus*, and *D. cynoscioni* from *Cynoscion regalis*, are all synonymous with *M. sagittatum*.

Hofer (1906) described and figured *Octobothrium sagittatum*. The mouth is shown as opening between, and posterior to, the oral suckers, i.e., it is placed definitely ventral some distance from the anterior extremity. The intestinal caeca appear to arise directly from the mouth, an oesophagus being entirely absent. Lühe (1909) also figures these organs in the same way.

We have no doubt whatsoever that both Hofer and Lühe have mistaken the pharynx for the mouth. The latter organ is very small and can only be seen properly with the oil immersion lens.

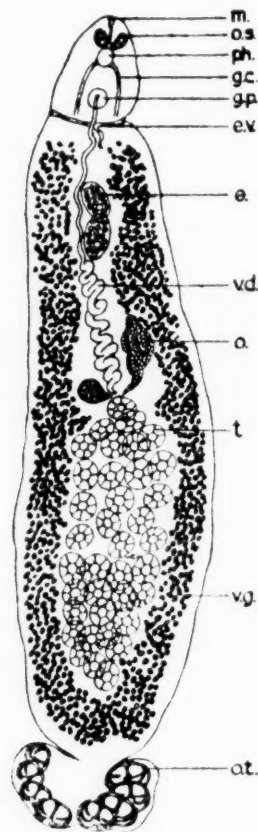


FIG. 3. *Mazocraes sagittatum*. ($\times 15$) (Lettering as in fig. 1.)

DESCRIPTION OF PARASITE

The worms are elongated and oval in shape, tapering towards the anterior extremity, which is bluntly rounded. Large forms measured 6 mm. in length by 1 mm. in breadth, and immature specimens 2 mm. by 0.35 mm. The two oral suckers are not very conspicuous, and are situated sub-terminally.

The mouth is small and terminal. The oesophagus runs posteriorly behind the oral suckers, and opens into a well-defined pharynx, which is about the same size as the suckers. The intestinal caeca arise from the lower end of the pharynx; they run close together, near the longitudinal axis, to the posterior extremity, ending just anterior to the organs of attachment. Two large excretory vessels, one running to each lateral margin of the body, practically separate the head from the rest of the body of the worm. They arise from a common longitudinal trunk in the middle of the body, and run transversely in opposite directions, each terminating at a well-developed pore.

Male genitalia. There are usually about 40 testes which are situated in the posterior half of the body. Each testis is more or less globular. In mature specimens the vas deferens is well developed and very convoluted. It runs anteriorly and opens at the genital pore. This is situated at the anterior extremity, immediately posterior to the mouth.

Female genitalia. The ovary and shell-gland are situated in the middle of the body, anterior to the testes. The uterus is a straight tube running from the ovary to the genital pore.

The vitellaria are composed of small separate follicles, abundantly scattered throughout the body and extending from the anterior transverse excretory vessels to the posterior extremity of the body.

In fully gravid specimens the eggs are few in number, never more than four being present in the uterus. These are oval, yellow in colour, and measure about 250μ in length by 130μ in breadth. They contain a large ovum surrounded by a mass of yolk.

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CORRIGENDUM

In a previous paper, 'A Description of a New Species of Amphistome, *Chiorchis purvisi*, with Notes on the Classification of the Genera Within the Group' (*Ann. Trop. Med. & Parasitol.*, **31**, 215-244), we omitted to observe that *Paracotyle* Johnstone, 1911, was an external parasite, and therefore not a digenetic trematode. We therefore withdraw the subfamily *Paracotylinae* and the genus *Paracotyle* from our classification of the *Paramphistomidae*.

STUDIES IN CHEMOTHERAPY*

XVI.—THE TRYPANOCIDAL ACTION OF SYNTHALIN

BY

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AND

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(Received for publication 5 September, 1937)

Recently, Jancsó and Jancsó (1935), and Schern and Artagaveytia-Allende (1935, 1936), showed that synthalin (decamethylene diguanidine) exerts a curative action on mice infected with various pathogenic trypanosomes. Both groups of workers were apparently led to make this interesting observation by the same considerations: viz., firstly, that trypanosomes require for their metabolism a large quantity of glucose; and secondly, that synthalin, when given in large doses, produces a pronounced hypoglycaemia.

The earliest observations on the influence of glucose on trypanosomes appear to be that of Biot, Biot and Richard (1911), who found that the length of time which *T. lewisi* survived in citrated blood at 17° C. was prolonged if glucose were added, and that of Fleig (1911), who showed the same thing in respect of *T. brucei*. The same year Schern observed that *T. equiperdum*, which had become motionless in preparations of citrated rat's blood, regained its motility when fresh blood or serum from a healthy rat or horse was added; he further noticed that liver emulsion exercised a remarkable life-prolonging action on the trypanosomes. This work of Schern's confirms the earlier work of Laveran and Mesnil (1904), who wrote 'Les Trypanosomes se conservent mieux, restent plus longtemps mobiles, dans le sang mélangé à du sérum que dans le sang pur. Nous avons vu des Trypanosomes encore mobiles, au bout de trois jours pleins, dans du sang de rat défibriné et mélangé à parties égales à du sérum de cheval; dans le sang pur, on ne trouvait plus, au bout de 24 heures, aucun Trypanosome mobile.'

After the Great War, Schern continued his work on this subject; and, in 1925, he published a series of papers in which he showed that the chief constituent of serum and liver responsible for the re-animation and prolongation of life of trypanosomes *in vitro* is glucose. He also found that liver extracts obtained from animals which have died of trypanosomiasis are deficient in yeast-fermentable bodies. As the result of this work Schern concluded that the blood- and liver-sugar is in some manner gradually used up by the trypanosomes in their metabolism. This process is at first regulated by the liver, which gives

*This work was assisted by a grant from the Chemotherapy Committee of the Medical Research Council.

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out into the blood a quantity of sugar corresponding to that consumed by the trypanosomes; but as the process continues, owing to the multiplication of the parasites, the liver finally becomes exhausted and almost entirely drained of sugar. One pathogenic result of trypanosomiasis is hence a disturbance of the liver function of storing carbohydrates; and finally there develops absolute hypoglycaemia and all its consequences.

During the last ten years a considerable number of papers dealing with this matter has appeared. Savino (1927) injected insulin into dogs infected with *T. equiperdum* and caused a diminution in the blood-sugar and also in the number of trypanosomes; the decrease in trypanosomes was believed to depend upon the hypoglycaemia, because a simultaneous injection of glucose and insulin resulted in only a slight diminution, if any, in the number of trypanosomes. Savino found, however, that the value of the glycaemia during the course of the infection did not bear a definite relationship to the number of trypanosomes in the blood. Regendanz and Tropp (1927) examined the behaviour of the blood-sugar in a series of rats infected with trypanosomes, and reached the conclusion that it is only shortly before death that the blood-sugar is markedly decreased in amount, and that the decrease is not due to consumption by the parasites, but to the effect of their toxins on the sugar-formation organs. Dubois and Bouckaert (1927) found that in the course of experimental trypanosomiasis in guinea-pigs and rabbits there is a notable hypoglycaemia; and Bruynoghe and these authors (1927) showed that the injection of insulin into an infected animal diminished the length of survival of its trypanosomes *in vitro*: they believed, however, that the hypoglycaemia in trypanosomiasis is not due entirely to consumption of glucose by the trypanosomes. Schern and Rossi-Lema (1928) recorded the occurrence of hypoglycaemia in chronic trypanosomiasis in the horse. Scheff (1928), who investigated the changes in the carbohydrate, protein and fat metabolism in rats infected with trypanosomes, found that the parasites primarily deprive the organism of carbohydrates and oxygen. As soon as the liver suffers secondary damage from over-work a state of hypoglycaemia arises. Many years previously Nauss and Yorke (1911) had drawn attention to the rapid disappearance of oxygen from blood to which trypanosomes were added, and they also pointed out that the increase in CO_2 did not correspond with the great loss of oxygen.

Yorke, Adams and Murgatroyd (1929), when elaborating their method for maintaining pathogenic trypanosomes alive *in vitro* at 37°C ., found that the presence of glucose was essential. They investigated the amount of glucose consumed by trypanosomes *in vitro*, and found that it was relatively enormous—400 million trypanosomes sufficing within 1 hour at 37°C . to cause the disappearance of between 2.0 and 2.5 mgm. of sugar, and within 5 hours of between 12.0 and 12.5 mgm. This observation has since been confirmed by Kligler, Geiger and Comaroff (1930), by Regendanz (1930), by Reiner and Smythe (1934) and by Jancsó (1935).

Regendanz (1929 and 1930) as the result of further experiments reached the conclusion that the blood-sugar value in rats infected with pathogenic trypanosomes is depressed only at the end of the disease. Infections of monkeys with *T. rhodesiense* were accompanied by a decrease of blood-sugar, but this was independent of the number of trypanosomes in the blood, and the blood-sugar was restored to normal by an injection of adrenaline. Regendanz re-states his earlier conclusion that the disturbance of sugar metabolism is most probably explicable by the toxic action of the trypanosomes on the organs of importance for sugar metabolism.

Subsequent observations of a somewhat similar nature by Zotta and Radacovici (1929), Angolotti and Carda (1929), Linton (1930), Locatelli (1930), Brand and Regendanz (1931) and Brand, Regendanz and Weise (1932), on the blood-sugar value in animals infected with trypanosomes, all gave the same general result, that pronounced hypoglycaemia only occurs in the agonal period of the disease or in the stage immediately preceding this.

Krijgsman (1933) examined the variations in the serum protein and blood-sugar titres of rats infected with *T. evansi*. No change in the blood-sugar value was noticed, except the well-known pre-agonal hypoglycaemia. Experiments were undertaken to ascertain whether altering the blood-sugar content, either in the positive direction by administration of adrenaline or glucose, or in the negative direction by insulin, had any influence on the infection: it was found that raising the blood-sugar value did not accelerate the development of the parasites, that there is always in the blood an amount of sugar in excess of that required by the trypanosomes, and that insulin has no effect on the development of the parasites.

Wormall (1932) carried out blood-sugar determinations in 12 sleeping sickness patients in Uganda. Whilst there was no general hypoglycaemia during the early stages of the disease, in certain instances a definitely low blood-sugar was obtained. Treatment with Bayer 205 produced variable results in the blood-sugar value, and no relationship could be established between the blood-sugar level and the presence or absence of trypanosomes in the blood. Glucose tolerance tests did not suggest that in human trypanosomiasis there was any marked impairment in the capacity of the liver to deal with glucose.

From all this work it seems possible to draw certain definite conclusions:—

1. The presence of glucose is necessary for the life of trypanosomes.
2. Trypanosomes in their metabolism consume relatively enormous quantities of glucose.
3. Laboratory animals experimentally infected with trypanosomes frequently exhibit, during the course of the infection, a certain degree of hypoglycaemia, but this is constantly present to a marked degree only in the agonal period of the disease or in the stage immediately preceding it.
4. The mechanism whereby the hypoglycaemia is produced is still uncertain.

Jancsó and Jancsó (1935c), having reached the conclusion that germanin acts by interfering with the carbohydrate metabolism of the trypanosomes—a conclusion based on the experimental observation that the changes in trypanosomes produced by germanin were similar to those resulting *in vitro* from the removal of sugar from the nutrient medium in which trypanosomes were suspended—decided to examine the action of the hypoglycaemia-producing guanidine derivatives* in experimental trypanosomiasis. They found that a number of these derivatives, especially synthalin, exerted a definite therapeutic action on mice infected with normal, arsenic-fast, and germanin-fast strains of *T. brucei*.

Having made this interesting discovery, the Jancsós pass to a lengthy consideration of the explanation of the phenomenon. They found that the changes produced in the trypanosomes by synthalin treatment were similar to those produced by germanin treatment, and consequently were, in their opinion, due to interference with carbohydrate metabolism. They then consider the question whether this action of synthalin is an indirect one dependent upon the fact that it produces a hypoglycaemia in the host, or whether it is a direct one due to the action of the drug on the parasite; and they advance a number of arguments—many of them based on purely hypothetical considerations—which appear to them to support one or other of these hypotheses.

Among the points supporting the theory of indirect action are :—

1. Trypanosomes disappear from the blood of infected marmots and dormice during hibernation (Blanchard, 1903; Blanchard and Blatin, 1907; Brumpt, 1908; and Vassiliadis and Jadin, 1930); and it has been shown that the blood-sugar value falls during hibernation (Feinschmidt and Ferdmann, 1932).

2. Jancsó suggests that the high virulence of trypanosomes for mice and rats may be associated with their particularly high blood-sugar.

3. The degenerative changes observed in trypanosomes during synthalin treatment is suggestive of an athreptic process. In the case of a drug (phenyl-

*In 1917, it was shown by Watanabe that the administration of guanidine HCl to rabbits is followed by hypoglycaemia, and this fact was subsequently confirmed by Collip (1923). Frank, Nothmann and Wagner (1926), making use of this observation, examined the effect of agmatine and of a series of methylene diguanidine compounds prepared by Kahlbaum, and found that several of them—notably synthalin (decamethylene diguanidine) and synthalin B (dodecamethylene diguanidine)—produced pronounced hypoglycaemia in normal rabbits when given in large doses. Dale (1927) reported that Bodo and Marks (1928), working in his laboratory, had found that in the rabbit the dose of synthalin necessary to produce hypoglycaemia was generally a toxic one; and they had reached the conclusion that the cause of the hypoglycaemia was damage to the liver. Cammidge (1927) stated that the results of his experiments with synthalin upon animals agreed in the main with those reported by Dale. He found, however, that even with therapeutic doses (1.5—2.0 mgm. per kilo.) hypoglycaemia was produced. With doses of 2 mgm. per kilo. an effect on the blood-sugar was seen, as a rule, in about 2 hours, but the maximum effect was not observed until about the 20th to 22nd hour; in fasting rabbits the blood-sugar had returned to normal by about the 30th hour.

arsenoxide) which acts directly, the metabolism of the parasite is deranged very rapidly, and the organisms disappear without undergoing any particular morphological change.

4. The fact that synthalin acts powerfully on the pan-fast (germanin-arsenic-fast) strain suggests an indirect action, because a characteristic of drug-fast trypanosomes is their impermeability to drugs : consequently, the action of synthalin cannot depend upon its penetration into the trypanosomes.

In Jancsó's opinion several considerations appear to favour the theory of direct action of synthalin, amongst which there are :—

1. It seems reasonable to expect that synthalin, the action of which is directed against the sugar metabolism of the cell, should have a pronounced toxic effect on an organism like the trypanosome, which has such an active carbohydrate metabolism.

2. If infected mice, treated with synthalin, are fed with bread, the treatment is tolerated without 'cramps,' and surely in these cases the hypoglycaemia must be but slight.

Summing up, Jancsó concludes that there is much to support the view that synthalin acts indirectly, the continuous hypoglycaemia resulting in a 'sugar-blockade' of the parasite metabolism.

Schern and Artagaveytia-Allende (1935 and 1936), who independently of Jancsó showed that synthalin has a definite therapeutic action on rats infected with *T. equinum*, also considered at length the mechanism of its action. They found that other substances which produce hypoglycaemia, viz., phosphorus, phloridzin, insulin and urethane, had no curative action on the trypanosomal infection, nor had starvation. They conclude, therefore, that the therapeutic action of synthalin is not solely due to the fact that it produces hypoglycaemia, although this is the chief cause, but is, in fact, also due to a toxic action upon the parasites. They describe synthalin therapy as 'glykoprive.' Schern believes that this 'glykoprive' action of synthalin in all probability finds its explanation in the peculiar behaviour of the drug on the carbohydrate-splitting ferments. This hypothesis is apparently based on the observations that synthalin increases the activity of the starch-splitting enzymes of the saliva, and that it at first increases and later inhibits the fermentation of sugar by yeast.

It appears from all this that Jancsó and Jancsó, and Schern and Artagaveytia-Allende, agree that synthalin exerts a curative action on mice infected with various pathogenic trypanosomes. They also agree in believing that the therapeutic action of synthalin is mainly due to the fact that it produces a hypoglycaemia ; but both groups of workers recognize the possibility that a direct action of the drug may play a subsidiary rôle in the process.

In view of the great interest of this discovery, we have considered it desirable to confirm the observations of Jancsó and Schern that synthalin has a definite therapeutic action on mice infected with pathogenic trypanosomes, and, having done so, to consider the manner in which the drug acts.

Our experiments on the therapeutic action of synthalin on mice infected with various pathogenic trypanosomes fully confirm those of Jancsó and of Schern. A single large dose (0.075 mgm. per 20 gm. mouse) produced a definite therapeutic action on mice infected with *T. equiperdum*, and on mice infected with the normal, the atoxyl-resistant and Bayer-resistant strains, respectively, of *T. rhodesiense*. With repeated doses of the drug it was found possible to cure mice in a certain proportion of cases.

TABLE I

Showing the trypanocidal activity of synthalin, synthalin B and insulin, respectively, on the normal strain of *T. rhodesiense*, suspended in guinea-pig serum-Ringer-glucose medium at 37° C.

Tube	Drug	Concentration of drug	No. of living trypanosomes per 256 squares of the haemocytometer scale		
			Start	After 6 hours	After 24 hours
1	Synthalin	1 : 4,096,000	100	0	
2		1 : 16,384,000		0	
3		1 : 65,536,000		4	0
4		1 : 262,144,000		98	4
5		1 : 1,048,576,000			96
6	Synthalin B	1 : 4,096,000		0	
7		1 : 16,384,000		0	
8		1 : 65,536,000		0	
9		1 : 262,144,000		76	6
10		1 : 1,048,576,000			84
11	Insulin	1 : 4,000*		0	
12		1 : 16,000		58	0
13		1 : 64,000			37
14		1 : 256,000			70
15		1 : 1,024,000			90
16	Control				94

*Calculated on the basis that a clinical unit contains 0.05 mgm. of insulin HCl.

In order to investigate the problem which has concerned so greatly the Jancsó and Schern and his collaborator—viz., whether the therapeutic action of synthalin is mainly indirect, through the hypoglycaemia which it produces, or whether the drug has a direct toxic action on the parasite—we examined its trypanocidal effect on suspensions of trypanosomes in nutrient medium (equal parts of guinea-pig serum and Ringer-glucose solution) at 37° C. *in vitro*, by the technique described in previous papers of this series (Yorke, Adams and Murgatroyd, 1929; and Yorke and Murgatroyd, 1930).

Experiments of this sort at once showed that synthalin *in vitro* was enormously toxic to our strains of *T. rhodesiense*, *T. equiperdum* and *T. brucei*, concentrations of only 1:65,500,000—1:262,000,000 sufficing to destroy all the parasites in the suspensions within 24 hours at 37° C.

Further experiments were then undertaken to compare the trypanocidal power *in vitro* of synthalin, synthalin B and insulin on the normal strain of

TABLE II

Showing the trypanocidal activity of synthalin, synthalin B and insulin, respectively, on the atoxyl-fast strain of *T. rhodesiense*, suspended in guinea-pig serum-Ringer-glucose medium at 37° C.

Tube	Drug	Concentration of drug	No. of living trypanosomes per 256 squares of the haemocytometer scale		
			Start	After 6 hours	After 24 hours
1	Synthalin	1: 4,096,000	100	0	
2		1: 16,384,000		0	
3		1: 65,536,000		19	0
4		1: 262,144,000		86	16
5		1: 1,048,576,000			76
6	Synthalin B	1: 4,096,000	100	0	
7		1: 16,384,000		0	
8		1: 65,536,000		4	0
9		1: 262,144,000		86	13
10		1: 1,048,576,000			84
11	Insulin	1: 4,000*	100	0	
12		1: 16,000		33	0
13		1: 64,000			29
14		1: 256,000			70
15		1: 1,024,000			86
16	Control				86

*Calculated on the basis that a clinical unit contains 0.05 mgm. of insulin HCl.

T. rhodesiense, and also on strains of this parasite which had been made completely resistant to atoxyl and to Bayer 205, respectively.* The results of these experiments are summarized in Tables I–III.

*The history of the normal strain and of the atoxyl-fast strain is given in Study XIV of this series (Murgatroyd and Yorke, 1937). The Bayer 205-fast strain was prepared recently by Hawking from our normal strain and is completely resistant to the gigantic dose of 2 mgm. per 20 gm. mouse, i.e., 80 times the dose which suffices to clear the blood of mice infected with the normal strain).

Tables I-III show that synthalin and synthalin B exert *in vitro* a powerful trypanocidal action, which is of the same order as that of the aromatic trivalent arsenical compounds (Yorke and Murgatroyd, 1930), a concentration of only 1 : 262,000,000 sufficing to destroy practically all the trypanosomes in a suspension within 24 hours. They further show that these drugs are just as active for the atoxyl- and the Bayer-resistant strains as they are for the normal strain. The trypanocidal action of insulin is relatively very slight, and no difference could be detected between its effects on the normal and on the resistant strains.

TABLE III

Showing the trypanocidal activity of synthalin, synthalin B and insulin, respectively, on the Bayer 205-fast strain of *T. rhodesiense*, suspended in guinea-pig serum-Ringer-glucose medium at 37° C.

Tube	Drug	Concentration of drug	No. of living trypanosomes per 256 squares of the haemocytometer scale		
			Start	After 6 hours	After 24 hours
1	Synthalin	1 : 4,096,000	100	0	
2		1 : 16,384,000		0	
3		1 : 65,536,000		26	0
4		1 : 262,144,000		74	7
5		1 : 1,048,576,000			70
6	Synthalin B	1 : 4,096,000		0	
7		1 : 16,384,000		0	
8		1 : 65,536,000		1	6
9		1 : 262,144,000		72	7
10		1 : 1,048,576,000			78
11	Insulin	1 : 4,000*		0	
12		1 : 16,000		12	0
13		1 : 64,000		46	31
14		1 : 256,000		104	90
15		1 : 1,024,000			88
16	Control				92

*Calculated on the basis that a clinical unit contains 0.05 mgm. of insulin HCl.

These *in vitro* results are in harmony with those obtained in infected mice, and afford an adequate explanation why synthalin and synthalin B exert a definite therapeutic action on mice infected with each of the three strains, whereas insulin fails to do so. They seem to warrant the conclusion that the therapeutic action of synthalin and synthalin B depends on a direct lethal effect of the drugs on the parasites.

The demonstration that synthalin and synthalin B exhibit a trypanocidal activity comparable with that of the aromatic trivalent arsenicals is of considerable academic interest, and possibly also of practical significance in that it encourages a search for similar or allied compounds of equal trypanocidal activity and of less toxicity for the host. A considerable series of such compounds has been prepared by Dr. H. King, of the National Institute for Medical Research, and the results obtained with these substances will be published in the near future.

SUMMARY

1. Attention is drawn to the recent interesting observation of the Jancsó and of Schern and Artagaveytia-Allende that synthalin exerts a curative action on mice infected with various pathogenic trypanosomes.

2. It is pointed out that the considerations which led these two groups of workers independently to make this discovery were apparently the same, viz., that trypanosomes require for their metabolism a large quantity of sugar, and that synthalin when given in large doses produces a pronounced hypoglycaemia. A summary of the literature bearing on these matters is given.

3. Reference is made to the fact that both Jancsó and Schern, as the result of a detailed consideration of the manner in which synthalin exerts its therapeutic action in experimental trypanosomiasis, reach the conclusion that it is mainly due to the fact that the drug produces a hypoglycaemia; both workers, however, recognize the possibility that a direct action of the drug on the parasite may play a subsidiary rôle in the therapeutic process.

4. Our experiments on the therapeutic action of synthalin on mice infected with various pathogenic trypanosomes confirm those of Jancsó and of Schern. A single large dose (0.075 mgm. per 20 gm. mouse) produced a definite therapeutic effect on mice infected with *T. equiperdum*, and on mice infected with the normal, the atoxyl-resistant and the Bayer-resistant strains of *T. rhodesiense*. With repeated doses of the drug it was found possible to cure a certain proportion of infected animals.

5. In order to throw light on the problem whether the therapeutic action of synthalin is due to a direct toxic action on the trypanosomes, or whether it is an indirect effect resulting from a hypoglycaemia, we have examined the trypanocidal action of the drug on suspensions of trypanosomes *in vitro* by means of the technique described in previous papers of this series.

It was found that synthalin (and synthalin B) exerts *in vitro* a powerfully trypanocidal action which is of the same order as that of the aromatic trivalent arsenicals, a concentration of only 1 : 262,000,000 sufficing to destroy practically all the trypanosomes in the suspensions within 24 hours. It was also found that synthalin is just as active *in vitro* for the atoxyl- and Bayer-resistant strains as for the normal strain.

The trypanocidal action of insulin *in vitro* is relatively negligible.

These observations seem to warrant the conclusion that the therapeutic action of synthalin depends upon a direct lethal effect of the drug on the parasite.

6. This demonstration of the powerfully trypanocidal action of synthalin has encouraged a search for similar or allied compounds of equal trypanocidal activity and of less toxicity for the host. The results obtained with a considerable series of such substances prepared by Dr. H. King will be published in the near future.

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SCREENCLOTH FOR HOUSES IN THE TROPICS

BY

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(Received for publication 15 September, 1937)

In a paper which I published (1935) under the above title, it was concluded that only two sizes of mesh and wire need be considered :

(a) For the screening of buildings, a 14-mesh screencloth made of wire of 30 Imperial Standard Wire Gauge.

(b) For the screening of water-containers which breed mosquitoes, an 18-mesh screencloth made of wire of 30 or 28 Imperial Standard Wire Gauge.

The selection of the 14-mesh was made in order to give the maximum amount of light and air compatible with what, at that time, appeared to be safety.

The Uganda Medical Report for 1935 records experiments with laboratory-bred adult females of *Anopheles funestus* : they failed to pass through the 14-mesh of 30 S.W.G. wire made of brass, and, as a result of these experiments by the entomologist, this mesh was recommended for use in the Uganda Protectorate.

It has, however, recently been reported to Professor Gordon by Dr. Rae of the Gambia that the 14-mesh screencloth lately installed in a hospital in the Protectorate does not exclude certain anophelines, which, it is suggested, may possibly be *A. funestus*. Professor Gordon has therefore carried out some experiments, and has found that ' a certain proportion of *A. funestus* and of *Stegomyia* are capable of passing through this gauze ' of 14-mesh made of wire of 30 Imperial Standard Wire Gauge.

In view of this experimental evidence from West Africa, it is clearly unsafe to employ the larger mesh mentioned, without experimental proof of its efficiency in each locality.

Arrangements are being made for local experiments in various parts of the tropics, and the results will be published in due course.

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PRINTED IN GREAT BRITAIN BY H. R. GRUBB, LTD., CROYDON.

ALLANTONEMA MUSCAE SP. NOV., A NEW
PARASITIC NEMATODE OF THE FAMILY
RHABDITIDAE FROM THE HAEMOCOELE OF
MUSCA VICINA

BY

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AND

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(Received for publication 26 July, 1937)

The nematode here described as a new species was discovered in the haemocoele of *Musca vicina*. When first seen, it was mistaken for *Habronema muscae*, the only species which has so far been described from house-flies; but when it was encountered a second time it was easily distinguished from *H. muscae*. In flies infected by this new species, adult females containing larvae and ova (unsegmented and embryonated) were found free in the abdomen of the host, while in *Habronema* infection only larvae were found, and they were mostly in the thorax, only a few being in the abdomen.

The larvae, which are often light brown in colour, are extremely active; they are 'rhabditiform' in type, and are present in very large numbers in the infected fly. Adults are generally few in number, not more than three or four being found in any single fly. Males have not been seen. The females are readily recognized by the presence of eggs in the uterus, which gives the creature an opaque white appearance. The adults are much less active than the larvae, and show no power of progression, their activity being confined to a lashing movement of the hinder part.

Out of nearly 1,500 stray flies dissected, the infection occurred in 5 specimens. Dissections of the fly were made in saline solution, and the nematodes when found were fixed in hot 70 per cent. alcohol.

The following is the description of the different parts.

ADULTS

MALE

None found.

FEMALE

To the naked eye it is opaque white. Its average length is 0.65 mm.; its average breadth is 0.12 mm.

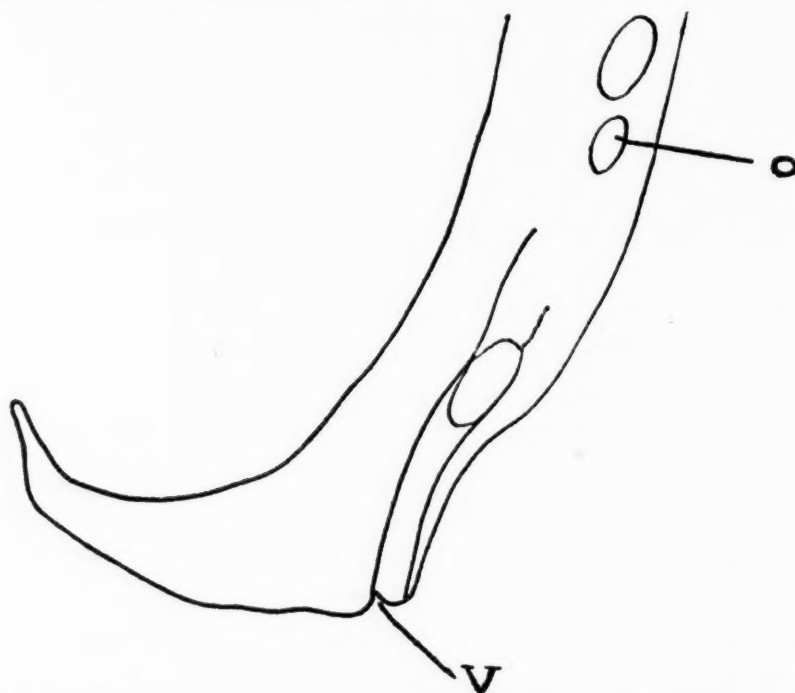
Head. Globular; at times a depression in the middle gives it the appearance of being bi-lobed; it is not marked off from the body; buccal capsule not present, and mouth absent.

Oesophagus. Not visible.

Tail. Very narrow and pointed at the end. Anal aperture not defined.

Vulva. Represented by two clearly defined lips opposite to each other and situated about 0.06 to 0.08 mm. from the terminal end.

Uterus. Only the terminal part of the uterine tube can, in a few specimens, be distinguished, and this portion contains eggs.



Camera lucida drawing of adult female *Allantonema muscae* sp. nov., showing the position of the vulva; the uterine tube is here visible only a short distance from the vulva. o.—Egg; v.—Vulva

LARVAE

The average length of the larva is 0.41 mm.; its average breadth is 0.017 mm.

OVA

Ova, unsegmented and embryonated, are found free in the body-cavity of the fly. Those found inside the uterine tube are unsegmented. The average measurements of eggs found free in the body-cavity are: length, 0.07 mm.; breadth, 0.03 mm.

INFECTION

The presence of larvae free in the body-cavity of the fly suggests that infection takes place in the larval stage, the infective larvae possessing the power of penetrating the gut wall of the adult fly and coming to lie free in its abdominal cavity, where further development to the adult stage takes place.

It is easy to distinguish larvae of *Habronema muscae* from those of *Allantonema muscae*. The former are much longer and broader, and the maximum number recorded in any single fly was about 20. Moreover, larval stages only are found, mostly in the thoracic and, less commonly, in the abdominal cavity of the fly.

DISCUSSION

The genus *Allantonema* was created by Leuckart (1884), while Baylis and Daubney (1926) regard it as belonging to Rhabditinae, greatly modified in consequence of specialism in habits.

On account of the absence of a mouth, a distinct anal opening and alimentary canal, this new nematode is placed under *Allantonema*.

There is a parasite species *A. mirabile* L. which has been described from the body-cavity of beetles. We have had no opportunity to consult the original descriptions and drawings of *A. mirabile* by Leuckart, and hence we cannot be certain if the new nematode is different from *A. mirabile*; but we have reason to believe that our specimen is not the same, since it has been discovered only in a particular species of house-fly and not in others which have very similar habits.

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**ALLANTONEMA STRICKLANDI SP. NOV., A
PARASITIC NEMATODE OF HOUSE-FLIES,
MUSCA VICINA**

BY

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AND

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(Received for publication 26 July, 1937)

We have described above (Roy and Mukherjee, 1937) a nematode, *Allantonema muscae*, from the body-cavity of *Musca vicina*, and we now propose to describe another nematode which has also been discovered in the body-cavity of the same fly. The chief points of distinction between *A. muscae* and this new species are its larger size, its more elongated shape, its rounded posterior end, its finely striated cuticle, the prominence of the uterine tube, and the presence of a characteristic papilla at the terminal extremity of the body. In other respects it resembles *Allantonema muscae*.

The specimens from which this type is described have been obtained from *Musca vicina* caught in the wild state. There were 5 adult female worms and a very large number of larvae and eggs (both unsegmented and embryonated), all lying free in the abdominal body-cavity. The adult females all possessed the same general features, but in only 3 out of the 5 were we able to demonstrate the presence of the terminal papilla. It can be seen under low magnifications.

DETAILED DESCRIPTION

Specimens were fixed in hot 70 per cent. alcohol.

ADULTS

MALE

None found.

FEMALE

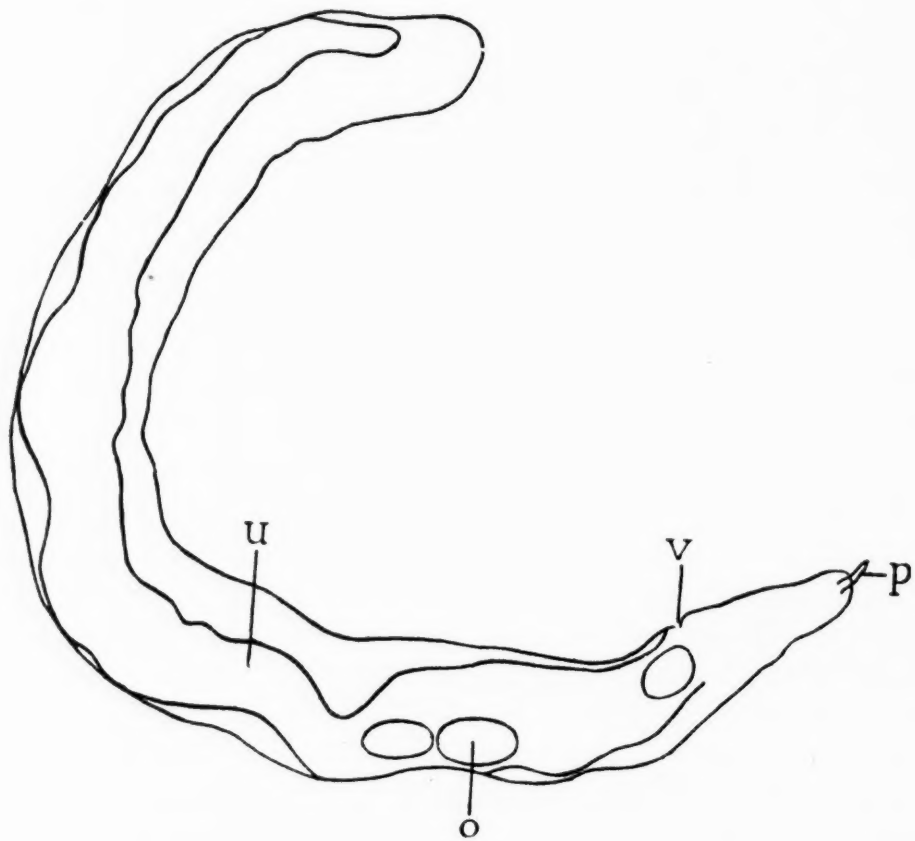
The average length of the female is 2.18 mm.; its average breadth is 0.2 mm.

Cuticle. Very finely striated; can only be seen under high magnification.

Head. Rounded; not demarcated from the body. Buccal capsule not present, and oral aperture not visible.

Oesophagus. Not visible.

Tail. Rounded at the posterior extremity, not pointed as in *A. muscae*. At the posterior extremity a terminal papilla is present, which measures about 0.027 mm. in length.



Camera lucida drawing of adult female *Allantonema stricklandi* sp. nov., showing the uterine tube which nearly fills the body. *u.*—Uterus; *o.*—Egg; *v.*—Vulva; *p.*—Terminal papilla.

Intestine. Indistinct.

Anus. Not visible.

Vulva. Represented by two clearly defined lips opposite to each other and situated from 0.12 to 0.25 mm. from the posterior extremity.

Uterus. Unlike *A. muscae*, the uterine tube is as a rule visible throughout the greater part of its length. The uterus begins near the anterior extremity of the worm and nearly fills the body. Inside the uterine tube eggs may be seen.

LARVAE

Larvae of all sizes are found free in the body-cavity; the size of mature larvae is as follows: maximum length, 0.5 mm.; maximum breadth, 0.026 mm.

EGGS

The eggs are oval; their length is 0.07 mm., and their breadth 0.04 mm.

DISCUSSION

In determining the systematic position of this nematode, we have been guided by its broad features and by its habits; and taking these into consideration we propose to place it in the family Rhabditidae and the subfamily Rhabditinae.

From the general point of view the main features of the adult and the organization of the parts suggest that it should be placed under the genus *Allantonema*. It differs from species of this genus, however, in one important respect: while in *Allantonema* most species are viviparous, the presence of eggs free in the body-cavity indicates that this new species of nematode is oviparous, like *Allantonema muscae*, and that the development of the embryo takes place outside the parent worm. Nevertheless, the absence of mouth, alimentary canal and anus, are strong points in favour of its inclusion in *Allantonema*.

This genus apparently belongs to the subfamily Rhabditinae Micoletzky, 1922.

The presence of a terminal papilla or a spike justifies its being considered as a new species, which we have pleasure in naming *stricklandi*, after Professor C. Strickland of this Institution.

The salient points of distinction between *Allantonema muscae* and *Allantonema stricklandi* are summarized below.

	<i>A. muscae</i>	<i>A. stricklandi</i>	Remarks
	ADULT		
Length	0.65 mm.	2.18 mm.	
Breadth	0.12 mm.	0.2 mm.	
Head	Anteriorly very broad.	Not so broad, rounded.	
Uterus	Not properly visible, except for that portion containing eggs. The anterior extremity is wholly indistinct.	Uterine tube prominent. Anterior end begins a short distance from the head.	
Vulva	0.07 mm. from the posterior extremity.	0.12-0.25 mm. from the posterior extremity.	
Posterior extremity of the body	Conical and pointed.	Broad and rounded, with a well-marked papilla.	
Cuticular striations	Not present.	Very fine transverse striations present.	

	<i>A. muscae</i>	<i>A. stricklandi</i>	Remarks
		LARVA	
Maximum length	0.5 mm.	0.5 mm.	No distinction can be made out between the larvae of the two species.
		EGGS	
Size	0.07 × 0.03 mm.	0.07 × 0.04 mm.	The eggs cannot be distinguished from one another.

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APPENDIX

Since writing the above note, we have encountered another *M. vicina* in which there were 3 adult *A. stricklandi* and numerous eggs free in the abdominal cavity; no larvae were found. The terminal papilla was present in each.

THE SYNONYMS OF THE TREMATODE GENUS *PACHYTREMA* LOOSS, 1907

BY

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(Received for publication 20 September, 1937)

Phadke and Gulati (1929) described a trematode found in the gall-bladder of *Corvus insolens* in India and named it *Multivitellaria hewletti* gen. et sp. nov., placing it in the family Dicrocoeliidae and erecting for it a new subfamily, Multivitellarinae.

The characters of the genus are : body thick and ventrally convex ; suckers close together ; pharynx and oesophagus present, caeca extending nearly to the posterior end of the body ; testes lateral, opposite, and in the posterior quarter of the body ; ovary post-testicular, median and slightly anterior to the fork of the excretory vesicle ; uterus pre-ovarial ; vitelline glands lateral to the caeca and extending from the acetabulum to the posterior extremities of the caeca ; genital pore close, but anterior, to the acetabulum ; pars prostatica and vesicula seminalis not enclosed by the cirrus sac ; excretory vesicle Y-shaped.

Though all these characters are not quoted by Phadke and Gulati, nevertheless they are all present (fig. 1).

The parasite differs from all known members of the family Dicrocoeliidae in that the uterus is anterior to the testes and ovary and that the excretory bladder is Y-shaped.

A brief summary of the distinguishing features of the families Dicrocoeliidae and Opisthorchiidae shows that *Multivitellaria hewletti* must be removed from the former to the latter family.

Dicrocoeliidae Odhner, 1910	Opisthorchiidae Braun, 1901
1. Excretory vesicle simple and tubular	Excretory vesicle usually Y-shaped
2. Cirrus sac present	Cirrus sac absent
3. Ovary usually behind testes	Ovary usually immediately in front of testes
4. Uterine coils behind testes and ovary	Uterine coils in front of testes and mostly in front of ovary

The excretory vesicle in *Multivitellaria hewletti* Phadke and Gulati is Y-shaped.

In their description of the latter species the authors state that the vesicula seminalis and pars prostatica are in close proximity to the cirrus sac. No figure

is given, but it is fairly evident that if a cirrus sac is present it must be rudimentary. In any case, in the Dicrocoeliidae the vesicula seminalis and pars prostatica are always enclosed in the cirrus sac.

In the Opisthorchiidae are included two species of the genus *Pachytrema*, viz., *P. magnum* Travassos, 1921, and *P. proximum* Travassos, 1921; in the latter species a rudimentary cirrus sac is present.

In *Multivitellaria hewletti*, *Pachytrema calculus* Looss, 1907, *P. magnum* Travassos, 1921, *P. proximum* Travassos, 1921, and *P. tringae* Layman, 1926, the arrangement of the gonads is identical, except that in some species the ovary lies approximately in the same transverse line and half way between the testes, whilst in others it lies slightly more posterior.

In reviewing the above list of characters of the families Dicrocoeliidae and Opisthorchiidae it will be seen that the only real point of differentiation is the relative position of the gonads to the uterus, taking into account the fact that in some genera of the Opisthorchiidae the excretory vesicle is not Y-shaped. The uterus is posterior to the gonads, which are in the anterior part of the body in Dicrocoeliidae, whilst in Opisthorchiidae the gonads are posterior and the uterus anterior. This means of differentiation has been overlooked by Phadke and Gulati. Multivitellarinae and *Multivitellaria* are then in the family Opisthorchiidae.

The Opisthorchiidae contains the subfamilies Opisthorchiinae Looss, 1899, and Pachytreminae Railliet, 1919.

Pachytreminae contains the genera *Pachytrema* Looss, 1907, *Diasia* Travassos, 1922, and *Microtrema* Kobayashi, 1915.

Multivitellaria differs from *Diasia* in that in the latter the gonads are lobulated and the suckers rudimentary and well separated. It differs from *Microtrema* in that in the latter genus the acetabulum is approximately in the middle of the length of the body. *Multivitellaria*, however, has no points of difference from *Pachytrema*, so that Multivitellarinae is a synonym of Pachytreminae, *Multivitellaria* a synonym of *Pachytrema*, and *M. hewletti* becomes *Pachytrema hewletti*.

Travassos (1928; footnote to p. 314) states that *Pachytrema magnum* was described by Linton in 1928 under the name *Minuthorchis sanguineus*, that Odhner found it to be identical with *P. calculus*, and that Pereira (1928) identified it as *P. magnum*.

The genus thus contains six species, viz., *calculus*, *hewletti*, *magnum*, *proximum*, *sanguineus* and *tringae*. The determination of their validity requires the concentration of the old and of any new material in one place, for study by sectioning to ascertain the true position of the organs; many of the apparent differences between the species may be due to differences of pressure during mounting. Looss, for instance, says that the length and breadth of *P. calculus* are almost doubled when the worm is mounted. In figures of *P. tringae*, the species, at first sight, appears to differ markedly from the other species in the

disposition of the vitelline glands; in the figure of *P. sanguineus*, particularly at one side, can be seen an approach to this merging into a single band of the groups of acini, which are well separated in the other species. In *P. hewletti* a small sucker surrounds the genital pore, and this appears to be the case also in *P. proximum* (figs. 1 and 2). In *P. sanguineus* the genital pore is, as I have already mentioned, situated anteriorly at the posterior edge of the oral sucker. It is more anterior than usual in *P. tringae* also. *P. calculus*, *P. hewletti*, *P. proximum* and *P. sanguineus* are broad forms, whilst *P. magnum* and *P. tringae* are narrower and more lanceolate in shape.

In short, the various species show an inter-grading and can be gathered

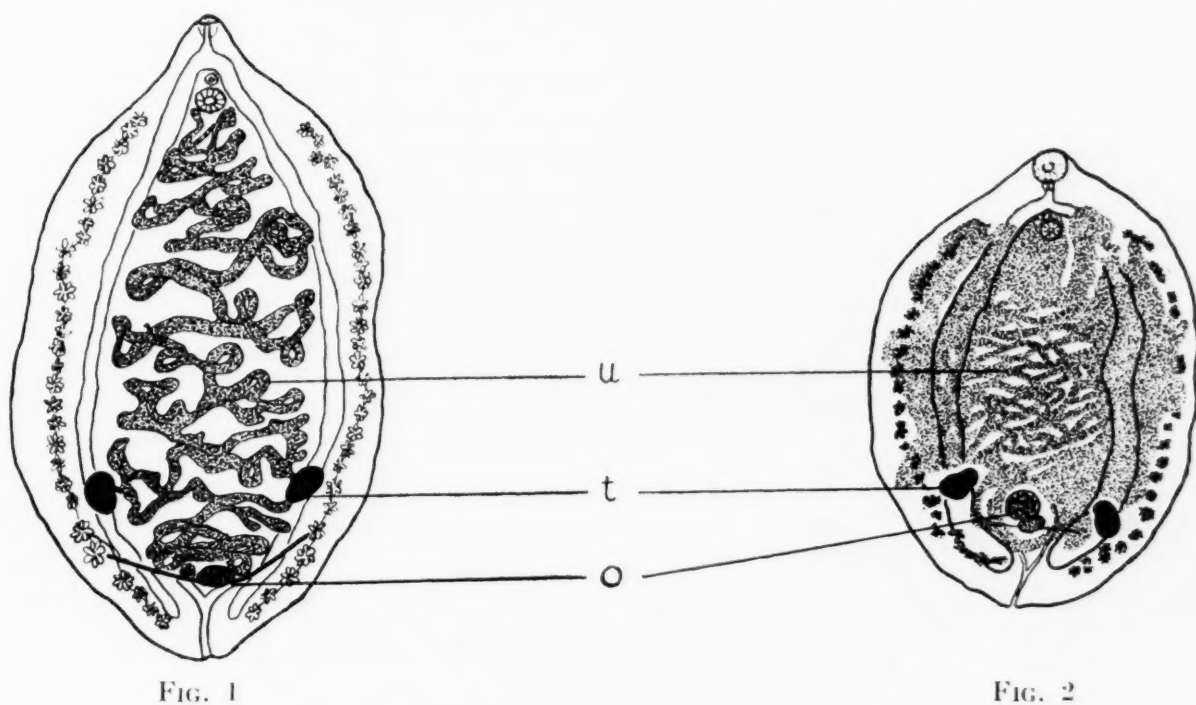


FIG. 1. *Multivitellaria hewletti* (after Phadke and Gulati).

FIG. 2. *Pachytrema proximum* (after Travassos).
u.—Uterus; t.—Testis; o.—Ovary.

into groups, the members of which vary according to the character chosen, in a manner suggesting that they really belong to the same genus and that no one of the six species now included is out of place.

SUMMARY

1. *Multivitellaria* Phadke and Gulati, 1929, is synonymous with *Pachytrema* Looss, 1907.

2. The relationship of the ovary to the testes cannot be used as a reliable character in distinguishing families of Distomata, though Pratt, 1902, Lühe, 1909, and Ward, 1918, all make use of this character.

3. In separating the families Dicrocoeliidae and Opisthorchiidae, the only reliable character is that in the former the uterus is posterior to the gonads and in the latter anterior to them.

4. *Minuthorchis* Linton, 1928, is a synonym of *Pachytrema*, but it is thought impossible to assign Linton's specific name as a synonym of any of the earlier ones.

5. Until the old material and any new material is thoroughly investigated by one worker, it is impossible to pass an opinion of any value on the validity of the six species now included in the genus, viz., *P. calculus* Looss, 1907, *P. hewletti* (Phadke and Gulati, 1929), *P. magnum* Travassos, 1921, *P. proximum* Travassos, 1921, *P. sanguineus* (Linton, 1928) and *P. tringae* Layman, 1926.

ACKNOWLEDGEMENTS.—I have pleasure in acknowledging a translation of the text and a copy of the figure of *P. tringae* from Dr. Mönnig; and my thanks are due to the Institute of Agricultural Parasitology and to the Director of Veterinary Services of the Union of South Africa for the loan of literature.

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STUDIES IN RURAL HYGIENE IN THE TROPICS

BY

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VI.—EDUCATION IN ELEMENTARY HYGIENE

(Received for publication 21 October, 1937)

Only forty years ago the work which lay before medical people in the tropics was research into the causes, the modes of spread, and the means which could prevent numerous unknown diseases; broadly speaking, that seemingly endless task has now successfully been accomplished, and the required knowledge exists. For the future, the chief difficulty which has to be overcome is to find some means of translating this knowledge into practice, and of putting within the reach of the people of the tropics those benefits in health which have thus become available for them.

The creation of sufficient health services, with adequate staffs of medical officers and sanitary inspectors, appears the obvious solution, but there are, as things stand, several grave impediments to it. The chief of these is the difficulty that, owing to the extra cost involved, such highly trained staff can never be provided on a scale large enough to cope with more than a fraction of the needs of the populations concerned, even in small and unusually prosperous parts of the Empire.

One useful preliminary towards the solution of the problem is the fact that most medical administrators in our tropical possessions have become inspired by the new ideal of prevention of disease in the community, in place of the old ideal of providing more hospitals and more dispensaries designed for the treatment of declared disease in the individual.

But, even given a determination on the part of medical administrators to concentrate the major part of their efforts on prevention, it is difficult to see how they can disregard the growing claims which existing disease makes upon their resources, or how they will succeed in their preventive aims with an inadequate sanitary staff.

In many tropical countries in which there exist serious but preventable diseases only a moderate amount of money is available annually for medical purposes, and the proper expenditure of this sum has become a matter of the greatest importance. If the prevention of disease can be attained only by the direct efforts of individual medical officers and sanitary inspectors, the prospects of rapid progress will be decidedly poor, because, so long as the cost of providing such medical and sanitary staff has to be borne by the people themselves, it will be difficult, owing to financial considerations, for the poorer colonies

to derive any great benefit, within a reasonable time, from the advances already made in the scientific knowledge of prevention of disease.

A policy which provides for any colony, protectorate or mandated territory only such health services for the prevention of disease as are of an expensive nature, is bound to have unfortunate results for impoverished populations. A system of this kind will naturally result in wealthy communities enjoying health services of a far higher standard than can be attained by those places which are without resources. Even if each colony and dependency were free to carry on the useful cultivation or development of its own particular products to the maximum extent, many of them could not provide medical and sanitary personnel on the scale required by them. Hence, if our colonies are to depend on their own financial resources for the means of diminishing their preventable diseases, it appears essential to aid them to reduce very substantially the expense of the methods employed.

Every medical worker in the tropics realizes that there are very many diseases now prevalent there which could largely be prevented if the people were properly educated to this end. By this is meant sufficiently educated to accept certain minimum standards of sanitation and to be willing to put into effect the measures necessary to attain them. Such an education involves theoretical and practical instruction in personal, house and village hygiene and also in dietetics.

In previous studies in this series, it has been emphasized that there are many diseases of the tropics in which a great deal of useful preventive work is of a simple and inexpensive nature. It has further been suggested that the people themselves could by their own efforts greatly diminish the incidence of these diseases and mitigate their evil effects. Their capacity to do this will depend on many economic considerations, but a prime necessity seems to be a thorough education in elementary hygiene.

It may be that certain education departments would regard without enthusiasm the prospects of their school-teachers becoming health mentors to their pupils. In places where this objection does not arise, however, if teachers can be trained in large numbers for rural school work, and can be shown that the health of their scholars and of their families is a primary concern to them as teachers, the prospects of achieving progress with regard to prevention of disease will be distinctly brighter. The school-teacher who will co-operate intelligently in work of this nature will have far-reaching influence, especially in the rural areas.

School-teachers called upon to impart instruction in hygiene to children will probably find it most convenient to do so in the form of a series of definite rules, particularly with regard to those matters where sound habit-formation is the ultimate aim. In order that the teachers may carry conviction, it is essential that they should have a clear understanding of the facts underlying the instruction in hygiene which they are giving to the children. This is not

to say, however, that all teachers should be expected to remember even as much as the names of the diseases which they will be so greatly instrumental in eradicating. They would, indeed, have difficulty in remembering these things, and fortunately it is not necessary to tax their brains with the effort to recall terms like elephantiasis, trypanosomiasis, and many other even more difficult examples of medical nomenclature. They will have their books on elementary hygiene beside them, and, if they like to refresh their memories from time to time out of interest in this side of the matter, well and good—that will be their private affair.

The highest grades of teachers—those, especially, who give instruction in teachers' training-colleges—will, without doubt, need some more detailed knowledge. They will probably require to know at least the names of the diseases, and to understand in a general way the mechanism of transference of the infection from one human being to another. But, passing down from the highest grades of the teaching staffs to the most junior, there will be a rapidly diminishing necessity for any detailed knowledge on these matters.

This can be exemplified by an item taken at random from the personal hygiene series of preventive measures. We may choose as an instance the necessity for attention to the hair. The small child is told to look after its hair, and is taught to consider a verminous state of it as socially undesirable; he or she is instructed how to prevent this condition, and how to get it cured should the necessity arise. The junior teacher must certainly be aware and convinced that, underlying the inculcation of this habit of attention to the hair, there is something more than a desire for aesthetic effect, there is the fear of definite and serious disease. But, beyond this conviction that hair-cleanliness is essential in the interest of health, the junior teacher's knowledge need hardly go. As mentioned above, it is not by any means necessary that the names or the causes of the diseases, far less the mechanism of transmission, should be remembered by such teachers, although they will have heard about these things in their training-colleges.

On the other hand, the most important work in hygiene devolving on the training-college instructors will be to convince the teachers in training of the need for scrupulous attention to the preventive measures prescribed. Insistence on this will bring home to them more than anything else a realization of the fact that a whole series of grave and incapacitating illnesses can be almost entirely avoided by the adoption of very simple preventive means. So elementary, indeed, are the means to be taken in many cases that it is more than probable that it will be difficult to convince teachers of their efficacy. Since the demonstrations of the actual methods of prevention are almost certain to seem the least impressive parts of the instruction from the recipient teachers' point of view, it is easy to understand that these methods may tend to be regarded somewhat casually. Yet it is just at this stage that the really capable and well-informed trainer of teachers will contrive to excel. Even the least inspiring

person can hardly fail to interest a group of teachers if he or she speaks with any clarity about the life-histories of the parasites of human disease during their migrations from one human being to another. The anxiety of the class to hear more about the biological phenomena which accompany the transference of diseases of man in the tropics will usually be sufficient to make a lecture or demonstration dealing with them a success. But when it comes to explanations of the means to be adopted for prevention, these will often sound so prosaic as to seem like bathos. Yet from the teacher-trainer's point of view the final and main object of the whole education of teachers in hygiene is to convey just this practical working knowledge regarding the methods of prevention which must be adopted. If the college trainers can fix indelibly on the minds of the teachers that it is the continuing practice of the appropriate preventive methods which really matters, and that this matters enormously, they will have the satisfaction of knowing that, by their work in their rural schools, the teachers whom they have trained will give a strong and lasting impetus to the improvement of the health of the community.

I believe that the conclusion from the facts is inescapable: that in great parts of our tropical and subtropical Empire much of the detailed instruction in practical elementary hygiene must finally be delegated to the local education authorities. It seems worth while, therefore, to try to discover what facilities exist at present for education in such localities, particularly what numbers of schools there are, and how many scholars attend them.

With this aim I have made a tabulation of the figures provided in the annual reports of the Education Departments of various colonies and dependencies. For the loan of many of these reports, and for kindly extracting for me information from others which were not available for loan, I am much indebted to the Librarian of the Colonial Office Library. The figures refer to education in government schools or in schools which are in some degree subsidized by the governments in the various colonies and dependencies. They do not give the complete educational picture, since many schools exist of which no figures are available; but they give a fair idea of what is being accomplished by various governments. In this connection it has to be borne in mind that the figures used for comparison, of approximately 15 scholars per cent. of the population of England and Wales, also do not represent the total scholars. For example, it is stated in the 1935 report of the Board of Education that in England and Wales there are large numbers of preparatory and secondary schools which are not grant-aided, and which have not applied to the Board of Education for recognition as efficient; these are not included.

The main results of the examination of the education figures for the colonies and dependencies are summarized in Table I.

According to the report of the Board of Education for 1935 referred to above, the percentage of scholars to population for England and Wales is 14.97. If we take this figure, say 15 per cent., as a standard, and apply it to the table

TABLE I

Colony or dependency	Population	Total no. of schools	Total no. of scholars	Scholars to population per cent.
Antigua	27,955	20	6,008	21.5
Bahamas	59,828	117	12,064	20.0
Barbados	182,440	137	26,668	14.63
Basutoland	500,000	519	64,381	12.87
Bermuda	16,436	30	2,545	15.48
British Guiana... ..	310,933	243	51,227	16.47
British Honduras	51,347	80	8,995	17.71
Brunei	33,648	19	1,214	3.6
Ceylon	5,312,548	5,211	705,196	13.26
Cyprus	358,466	884	52,902	14.75
Dominica	36,503	29	7,683	21.04
Falkland Islands	3,101	4	392	12.64
Federated Malay States	1,706,746	1,311	107,048	6.28
Fiji	193,268	312	20,417	10.56
Gambia... ..	199,292	16	2,194	1.0
Gilbert and Ellice Islands	33,469	130	6,924	20.68
Gold Coast	3,161,084	676	62,787	1.67
Grenada	66,302	64	13,778	20.78
Hong Kong	821,429	1,054	70,853	8.62
Jamaica	1,104,775	671	150,134	13.58
Kedah	429,280	96	11,081	2.58
Kelantan	368,287	58	4,863	1.32
Kenya	3,078,908	1,540	104,061	3.05
Mauritius	377,572	135	41,037	10.86
Montserrat	12,008	12	2,839	23.55
Nigeria (Northern and Southern Provinces)	19,452,995	3,536	216,572	1.06
Northern Rhodesia	1,367,026	1,603	87,119	6.37
Nyasaland	1,599,888	3,318	169,087	10.57
Palestine	1,194,529	998	102,718	8.6
Penang	340,140	20	8,667	3.6
Perlis	51,101	24	2,411	4.71
St. Kitts and Nevis	32,859	31	7,514	22.86
St. Vincent	55,219	37	10,432	18.9
Sierra Leone Colony and Protectorate	1,768,480	246	18,643	1.05
Straits Settlements	1,104,012	340	60,173	5.44
Swaziland	110,746	12	4,803	4.33
Tanganyika Territory... ..	5,046,062	4,954	229,879	4.55
Tonga Island	30,482	118	7,828	25.67
Trengannu	179,754	28	2,542	1.41
Trinidad and Tobago	412,783	229	72,639	17.6
Uganda... ..	3,638,677	6,169	229,647	6.31
Virgin Islands	5,026	10	1,252	24.91
Zanzibar	235,150	33	4,067	1.72

of colonies and dependencies, we find a surprisingly large number of them with a percentage as high or even higher.

It will be seen from Table I that the following have a percentage of scholars to population equal to or greater than the figure for England and Wales for 1935: Antigua, Bahamas, British Guiana, British Honduras, Grenada, Montserrat, St. Kitts and Nevis, Tonga Island, Trinidad and Tobago, and the Virgin Islands. However, it will be noted that the populations of these places are very small, none of them, with the exception of those of British Guiana and Trinidad with Tobago, being over 66,000.

In Table II the countries are arranged in descending order of size of population.

Among countries with a population of three millions or over, the percentage of scholars to population varies from more than 13 per cent. in the case of Ceylon (population over five millions), to just over 1 per cent. in the case of Nigeria (population over nineteen millions). In the group of countries with over one million of population but less than three millions, there appears a similar range, Jamaica having more than 13 per cent. of scholars, while Sierra Leone has just over 1 per cent.

From these tables it will be noted that the West African colonies stand out conspicuously on account of their strikingly low percentages of scholars: Nigeria (population over nineteen millions) 1.06 per cent.; Gold Coast (population over three millions) 1.67 per cent.; Sierra Leone (population over one million) 1.05 per cent.; the Gambia (population two hundred thousand) 1 per cent. These countries rank educationally with Kelantan, Trengganu and Zanzibar.

It is not easy to determine what factors are responsible for the remarkable differences that appear in the school educational status of the various countries. The inclusion of schools which do not come under the heading of government or government-aided would doubtless have some effect in raising the lower percentages.

An attempt has been made to ascertain whether the age of the colony or dependency may have had an effect, in that the older possessions have advanced further than the more recent acquisitions. For this purpose there are shown in Table III six of the countries having high percentages of scholars (*a*), and six of those having low percentages (*b*), arranged in descending order of percentages, together with the date at which the country came into our possession.

In some of the instances it has not been an easy matter to determine the exact age of the possession. For example, in the case of Sierra Leone, a hundred and fifty years have elapsed since Freetown was founded, while the protectorate was acquired forty years ago. There are cases also in which the date given does not represent the earliest British occupation—for example, Dominica, a possession which passed from the French to the British and back again several times before its final acquisition by Britain. Among the six

TABLE II

Colony or dependency	Population over	Scholars to population per cent.
Nigeria	19 millions	1.06
Ceylon	5 ..	13.26
Tanganyika Territory	5 ..	4.55
Kenya	3 ..	3.05
Gold Coast	3 ..	1.67
Uganda	3 ..	6.31
Straits Settlements	1 million	5.44
Jamaica	1 ..	13.58
Palestine	1 ..	8.6
Northern Rhodesia	1 ..	6.37
Nyasaland... ..	1 ..	10.57
Federated Malay States... ..	1 ..	6.28
Sierra Leone	1 ..	1.05
Hong Kong	800,000	8.62
Basutoland	500,000	12.87
Trinidad and Tobago	400,000	17.6
Kedah	"	2.58
British Guiana	300,000	16.47
Penang	"	3.6
Cyprus	"	14.75
Kelantan	"	1.32
Mauritius	"	10.86
Zanzibar	200,000	1.72
Swaziland	100,000	4.33
Trengannu	"	1.41
Barbados	"	14.63
Fiji	"	10.56
Gambia	"	1.0
Grenada	66,000	20.78
Bahamas	59,000	20.0
St. Vincent	55,000	18.9
Perlis	51,000	4.71
British Honduras	"	17.71
Dominica	36,000	21.04
Gilbert and Ellice Islands	33,000	20.68
Brunei	"	3.6
St. Kitts and Nevis	32,000	22.86
Tonga Island	30,000	25.67
Antigua	27,000	21.5
Bermuda	16,000	15.48
Montserrat	12,000	23.55
Virgin Islands	5,000	24.91
Falkland Islands	3,000	12.64

countries with high percentages of scholars, the date of acquisition varies, as will be seen from the table, from 1666 to 1900 ; in the six showing low percentages of scholars the date of acquisition varies from 1788 to 1909. A peculiar point which may be noted is that in group (a) Tonga Island has the highest percentage ; yet this was actually the most recently acquired possession in that group, dating only from 1900.

TABLE III

Country	Population	Scholars to population per cent.	Date of colony or dependency
(a)			
Tonga Island	30,482	25.67	1900
Virgin Islands	5,026	24.91	1666
Montserrat	12,008	23.55	1783
St. Kitts and Nevis ...	32,859	22.86	1783
Antigua	27,995	21.5	1666
Dominica	36,503	21.04	1833
(b)			
Gold Coast	3,161,084	1.67	1871
Trengannu	179,754	1.41	1900
Kelantan	368,287	1.32	1909
Nigeria	19,452,995	1.06	1900
Sierra Leone	1,768,480	1.05	Colony, 1788 ; protectorate added, 1896
Gambia	199,292	1.0	1829

In presenting this account of the educational facilities provided by government for people in countries where tropical diseases are common, I am actuated by the belief that a suitable type of education is essential for any tropical country if we aim at even a moderately rapid alleviation of the bad health conditions which admittedly exist.

If this belief is well founded, it follows that adequate means of acquiring the elementary education necessary should be placed within the reach of the peoples of those countries which are backward in this respect.

THREE NEW SPECIES OF *CULICOIDES* (DIPTERA, CERATOPOGONIDAE) FROM MALAYA

BY

J. W. S. MACFIE

(Received for publication 19 November, 1937)

I have been privileged to examine the collection of *Culicoides* made in Malaya by Mr. J. J. Buckley in the course of his recent work on onchocerciasis. Three of the species appear to be new, and are described below. In addition, the collection contained specimens of the following species: *Culicoides anophelis* Edw. (15 ♀♀); *C. buckleyi* Macfie (2 ♀♀); *C. daleki* Smith and Swaminath (3 ♀♀); *C. gentilis* Macfie (2 ♀♀); *C. orientalis* Macfie (18 ♂♂, 35 ♀♀); *C. oxystoma* Kieff. (4 ♂♂, 13 ♀♀); *C. peregrinus* Kieff. (3 ♂♂, 11 ♀♀); *C. pungens* de Meij. (45 ♀♀); *C. raripalpis* Smith (61 ♀♀); *C. shortti* Smith and Swaminath (10 ♀♀); *C. similis* C.I. and M. (1 ♀); and *C. sumatrae* Macfie (11 ♀♀). All the specimens were taken at Kuala Lumpur in 1936 or 1937.

It is possible that more than a single species may be included under each of the names *C. orientalis*, *C. pungens* and *C. raripalpis*; but it would be inadvisable, I think, to attempt to separate them until the range of variability of the species is better known, and (in some cases) until males are available for examination. Some of the specimens, referred to as *C. raripalpis*, however, have at the tip of the wing a rather distinctive pale band not shown in Smith's (1929) figure of the wing of this species; the pale area covering the end of the costa is rather larger, and in consequence the dark area proximal to it is rather smaller. These specimens may be of a distinct new species. But, as they otherwise agree in morphological characters with *C. raripalpis*, and in particular have spermathecae of the same unusual shape, it would be best I think, to regard them only as a variety, unless males, when procured, show distinct differences.

C. oxystoma Kieffer, 1910, does not differ apparently in any essential respect (including the characters of the hypopygium) from the common African species *C. schultzei* End., 1908.

***Culicoides insignipennis* sp. nov.**

A very dark brown species, with wings adorned with numerous pale spots, two of which are situated between the branches of Cu.

♀. Length of wing, about 1.2 mm.; greatest breadth, about 0.5 mm.

Head very dark brown, blackish. *Palpi* very dark brown, third segment only slightly inflated, with a small shallow pit just beyond the middle; lengths

of last three segments about 20, 12 and 11 units* respectively. Antennae dark brown: segments 4-10 somewhat vasiform, measuring in one specimen from 11 by 6 to 15 by 5 (maximum) units; 11-15 rather more elongate, 11-14 from 17 to 20 by 4-5 units, 15 about 26 by 5 units, with a rather long terminal process. The combined lengths of segments 3-10, 4-10 and 11-15 about 100, 88 and 98 units respectively.

Thorax very dark brown, mottled. Scutellum blackish, bearing the usual 3-4 bristles, but no small hairs.

Wings rather dark. Adornment as shown in the diagram (fig. 1). The

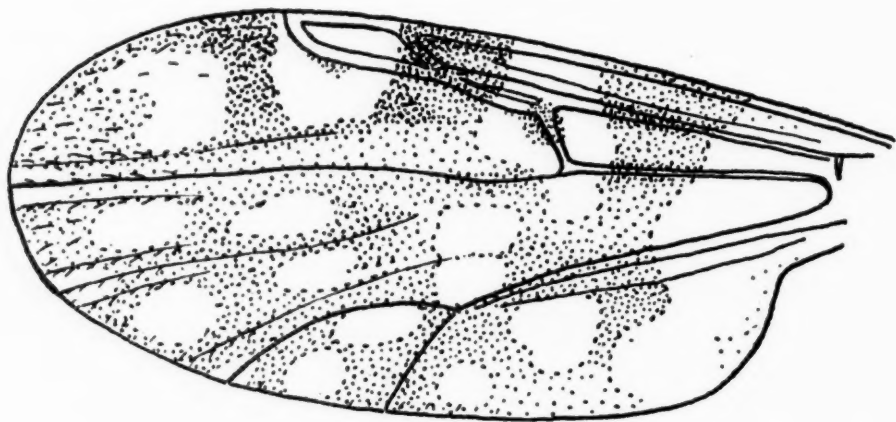


FIG. 1. *Culicoides insignipennis* sp. nov. Wing of female.

most notable features are the two pale spots between the branches of Cu and the dark spot in the middle of the pale area which covers the cross-vein. Second radial cell rather long, but not unusually broad. Macrotrichia scanty, limited to the distal third of the wing. Halteres with dark-brown knobs.

Legs very dark brown. Knees paler, yellowish, especially on the hind legs. Hind tibiae narrowly yellow at base and apex. T.R. about 2. Fourth tarsal segments not cordiform.

Abdomen very dark brown, tip and cerci pale brown. Spermathecae two, highly chitinized, dark in colour, oval but tapering slightly towards the duct, not quite equal, about $50-60\mu$ by $35-40\mu$. Practically no part of the duct is chitinized.

MALAYA: Kuala Lumpur, 1937, 4 ♀♀ (J. J. Buckley).

This species differs from all others with which I am acquainted in the adornment of the wings, particularly in having two pale spots between the branches of Cu.

*The unit used is 3.7μ .

Culicoides malayae sp. nov.

A dark-brown species, with wings adorned with numerous pale spots, one of which lies immediately anterior to the fork of Cu and just covers the angle formed by the bifurcation of this vein.

♀. Length of wing, about 1.4 mm.; greatest breadth, about 0.6 mm.

Head very dark brown. Eyes contiguous above. Palpi dark brown, third segment only slightly inflated, with a small, shallow pit in anterior half: lengths of last three segments about 21, 12 and 10 units respectively. Antennae dull brown, the basal segments paler at bases: segments 4–10 somewhat drawn-out distally, ranging from about 12 by 6 to 13 by 5 units; 11–15 more elongate, about 17, 17, 22, 23 and 33 by 5 units. The combined lengths of segments 3–10, 4–10 and 11–15 about 124, 108 and 112 units respectively.

Thorax dark brown, with paler brown bands on the middle of dorsum and at sides. Scutellum dark brown, bearing four bristles and two small hairs.

Wings with adornment as shown in the diagram (fig. 2). The pale spot in

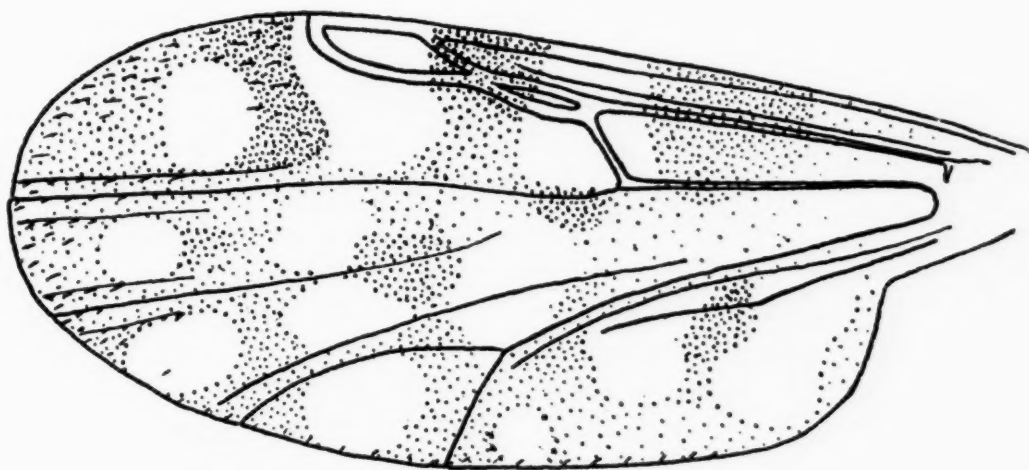


FIG. 2. *Culicoides malayae* sp. nov. Wing of female.

the middle of the wing extends posteriorly to the fork of Cu and just covers the angle formed by the bifurcation of this vein. Macrotrichia scanty, restricted to the distal third of wing. Halteres with white knobs.

Legs dark brown. Hind legs with femora entirely dark brown, tibiae with narrow yellowish band at base and apex. T.R. about 2. Fourth tarsal segments not cordiform.

Abdomen dark brown, but tip and cerci pale. Spermathecae two, well chitinized, obovate, not quite equal, about 55μ by 40μ . The duct is narrow, and chitinized for only a short distance, about 3μ .

MALAYA : Kuala Lumpur, 1936, 1 ♀ (J. J. Buckley).

In my key (1937) for the determination of species of *Culicoides* found in Malaya this insect requires to be distinguished from *C. peregrinus*. This may readily be done by the fact that the pale spot in the middle of cell R_5 does not reach the anterior margin of the wing.

***Culicoides geminus* sp. nov.**

This species resembles very closely *C. daleki* Smith and Swaminath, but has two spermathecae. They are well chitinized, obovate, sub-equal, about 46μ by 37μ , with almost no part of the duct chitinized. The only other difference which I have been able to detect in the specimens at my disposal is that in this species the pale spot on the wing which envelops the cross-vein is rather larger, and covers almost the whole of the first radial cell, whereas in *C. daleki* it covers only about half this cell.

MALAYA : Kuala Lumpur, 1937, 2 ♀♀ (J. J. Buckley).

OBSERVATIONS ON THE THERAPEUTIC ACTION OF THREE ARSENICALS, NEOCRYL, K.324 AND K.352, IN GAMBIAN SLEEPING SICKNESS

BY

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(*Received for publication 29 September, 1937*)

Several new compounds, synthesized by workers under the Chemotherapy Committee of the Medical Research Council, have been found to have considerable trypanocidal activity, and preliminary experiments with one of these indicated that it might have a definite therapeutic value in human trypanosomiasis and syphilis. Consequently it seemed desirable to extend the trial of these preparations, and the following paper records observations made in the Gambia, West Africa, on the therapeutic action of three of these drugs—neocryl, K.324 and K.352—in sleeping sickness.

NEOCRYL

This compound, sodium succinanimomethylamide-p-arsonate, prepared by Morgan and Walton, is a pentavalent arsenical of the formula $\text{NaO.HO.OAs} \cdot \text{NHCO} \cdot (\text{CH}_2)_2 \text{CO.NHCH}_3$. It is a white crystalline substance readily soluble in water, and resembles tryparsamide in its toxicity and trypanocidal action on experimental infections in laboratory animals. These observations have already been described at length by Yorke and Murgatroyd (1936), who, with others, also examined its therapeutic action in syphilis in man, where it was found to have a definite therapeutic action at all stages of the disease. It has since been tried in syphilis by a number of workers on behalf of the Therapeutic Trials Committee of the Medical Research Council, with results (unpublished) which so far have been less encouraging. The drug was also used by Lester (see Yorke *et al.*, 1936) in 12 cases of Nigerian sleeping sickness with promising results. Acres (1937) has recently recorded his observations on a series of 24 cases of sleeping sickness treated with this drug at Bolobo in the Belgian Congo. Three of these cases had previously proved unresponsive to arsenicals and can be disregarded. Dealing with the remainder, he found that in first-stage cases there was definite clinical improvement, the immediate effects, as determined by gland and lumbar puncture, were satisfactory, and the results clinically and from the laboratory point of view compared favourably with those obtained with tryponarsyl, the Belgian equivalent of tryparsamide. In second-stage cases, despite marked clinical improvement, the immediate results were not so satisfactory, and in his opinion did not compare so favourably with those obtained by tryponarsyl. He did not think that with neocryl the reduction of the cell count of the cerebrospinal fluid was so marked, nor did it occur as regularly as in his cases treated with tryponarsyl. In three instances

the drug failed, one case remaining positive at the end of treatment and two others quickly relapsing. Acres points out that patients in his district occasionally remain positive after a full course of trypanarsyl, but he is of the opinion that the relapses occurred more quickly after neocryl than they would have done after trypanarsyl. One of the two cases relapsing was, however, an old case of sleeping sickness that two years previously had been treated with trypanarsyl, and it is difficult to be sure whether in this instance neocryl was being tested against a true reinfection or against a late relapse following the original treatment with trypanarsyl. In only one patient did he find any suggestion of a toxic reaction. This occurred in an old case, who was considered to be unresponsive to trypanarsyl, since his condition was deteriorating under the treatment with this drug, and who was given 4 gm. of neocryl weekly. After five injections he suffered from dimness of vision, which showed no improvement three months after the drug was stopped.*

PRESENT SERIES

In the present series, every patient was given a full clinical examination before treatment, and a careful note was made of the state of the vision and the appearance of the fundi. Blood, gland-juice and the centrifuged deposit of the cerebrospinal fluid were examined for trypanosomes, and the cell count and protein content of the cerebrospinal fluid were also determined.† No case was treated as sleeping sickness unless trypanosomes were seen.

The examinations were repeated occasionally during the course of treatment, at the end of treatment, and, where possible, at intervals subsequently.

One hundred and twenty-two cases were treated, of which 44 had normal cerebrospinal fluids and 78 pathological fluids. As a routine, 10 doses of 0.045 gm. per kgm. body weight were given at weekly intervals, but many of the patients attended irregularly, and in a few cases higher doses up to 0.06 gm. per kgm. body weight were given; these deviations are recorded in the tables. The drug was administered intravenously in 25 per cent. solution in freshly distilled water.

*In an addendum to his paper Acres recently states: ' . . . before 6 months had elapsed after their courses of treatment . . . of nine first-stage cases, two have had to be taken under treatment again because of raised cell count in the cerebrospinal fluid. Of twelve second-stage cases, either seven or eight relapsed and have been taken under treatment again.'

†Five cases were treated without preliminary examination of the cerebrospinal fluid, and have consequently been disregarded. Three of these absconded, clinically improved, after a few doses of neocryl, while another, after a single dose, was accidentally treated with a course of tryparsamide. The fifth, a very excitable patient, disappeared after the second dose of neocryl, and 13 days later was found wandering and completely mentally deranged. Treatment was reinstituted, but although he improved considerably he was still emotionally unstable at the end of the course. The spinal fluid was then examined, and was found to be straw-coloured, with a protein content of 2 per cent. and a cell count of 150 white cells per c.cm. and 1,800 red cells per c.cm.; no parasites were seen. The facts suggested something in the nature of a Jarisch-Herxheimer reaction following the second dose of neocryl, comparable to that of a case treated with tryparsamide reported fully by Mackie (1935).

CASES WITH NORMAL SPINAL FLUIDS

The results of treating these cases are summarized in Table I (pp. 476-491), from which the following facts emerge.

The total cases treated numbered 44, of which 30 received a course of 10 doses. All these 30 cases were clinically improved, and in every instance the cerebrospinal fluid remained normal and the gland-juice and blood, where originally positive, became negative at the end of treatment. Early in the course of treatment the general condition improved remarkably, symptoms disappeared, and in most cases there was a steady gain in weight. Of the 14 cases absconding before the end of treatment, all appeared clinically well or very much improved at the time of their disappearance.

Toxic Effects. Two patients (Cases 45 and 104) suffered definite visual failure, but both recovered. Case 45 was absent for six weeks after the first dose of neocryl, and on returning reported that the absence had been due to failure of vision. Similarly, Case 104 disappeared for six weeks after the third dose, and on returning stated that vision had failed for 10 days after the third dose and had then slowly returned. At the time of re-examination no visual abnormality was apparent in either patient. The first patient was given a full course of treatment without any further eye trouble, while the second patient continued treatment without showing any sign of visual disturbance for two weeks after her return, and then absconded apparently well.

Relapses. Although no case failed to respond to treatment, and there were no relapses as judged by the reappearance of trypanosomes, one patient (Case 12) presented symptoms and signs of a relapse. This patient appeared quite well at the end of treatment and also 11 weeks later, when the protein content and cell count of the spinal fluid were 0.020 per cent. and 4 per c.mm. respectively. Sixteen weeks after finishing treatment she complained of occasional headaches, and the protein content and cell count of the spinal fluid were then 0.110 per cent. and 40 per c.mm. respectively, although no trypanosomes could be found.

Two other cases are of interest. One of these (Case 5) had absconded after nine doses, apparently quite well; seven weeks after the last dose he was seen again, when he was still clinically well, and no parasites could be found, but the protein content and cell count of the spinal fluid were 0.060 per cent. and 165 per c.mm., whereas the figures before treatment were only 0.030 per cent. and 4 per c.mm. respectively. Another patient (Case 73), originally suffering from headaches and with positive glands, absconded after a single dose, but was re-examined 18 weeks later, when she stated that she was quite well and free from headaches. She had, however, lost a considerable amount of weight and had a very marked tachycardia, which suggested, as one might expect, that the disease was still active. Nevertheless, careful examination of the gland-juice failed to reveal trypanosomes, and the spinal fluid was normal in all respects.

TABLE I.—Showing the results of treating patients (with normal cerebrospinal

Case	Condition immediately before treatment							Treatment. Gm. neocryl per kgm. body weight × no. of doses. Interval between doses
	Clinical	Body weight in lb.	Blood tryps.	Gland- juice tryps.	C.S.F.			
					Protein %	Cells	Tryps.	
5	4 months. Headaches and somno- lence. Moderate- to large-sized glands in neck and axillae. Pulse 96.	138	—	+	0.030	4	—	0.045 × 9 weekly (2 weeks between 2nd and 3rd dose)
12	1 year. Headaches, no somno- lence. Small glands in neck and axillae. Pulse 112.	90	—	+	0.035	2	—	0.075 × 10 weekly (2 weeks between 1st and 2nd dose)
14	6 months. Glands in neck. 2 months. Headaches and somno- lence. Large glands in neck and axillae. Pulse 88.	138	—	+	0.025	4	—	0.045 × 10 weekly (Absent 3 weeks between 1st and 2nd dose)
15	3 months. Generalized pains, absent-mindedness. Glands in neck. No somnolence. Gen- eralized adenitis. Pulse 120.	55	—	+	0.020	1	—	0.045 × 1

Condition after treatment								
Time of examination	Clinical	Body weight in lb.	Blood tryps.	Gland-juice tryps.	C.S.F.			Remarks
					Protein %	Cells	Tryps.	
7 weeks after 9th dose	Appears quite well. No headaches or somnolence. Glands much smaller. Vision normal. Pulse 100. Urine n.a.c.	136	—	—	0.060	165	—	(Absconded after 9th dose apparently quite well.) Clinically well, but C. S. F. now abnormal.
At 10th dose	Appears and feels quite well. Glands very small on left side neck and almost disappeared from right side. Vision normal. Pulse 78.	100	—	—	0.030	5	—	
6 weeks after 10th dose	Quite well. Pulse 102. Glands too small to puncture.	102						
11 weeks after 10th dose	Still quite well. Pulse 98. Glands too small to puncture.	98	—		0.020	4	—	
16 weeks after 10th dose	Occasional headaches, otherwise normal. Reflexes and vision normal. Pulse 84. Glands too small to puncture.	105	—		0.110	40	—	Occasional headaches and abnormal C.S.F. suggest relapse, although no parasites were found.
At 10th dose	Appears and feels well. Glands diminished in size. Vision normal. Pulse 84.	141	—	—	0.025	3	—	
6 weeks after 10th dose	Still apparently quite fit. Pulse 96.	132	—	—	0.030	4	—	Apparently quite well, but has lost a little weight.
								Absconded after 1 dose.

TABLE I (Continued).—Showing the results of treating patients (with normal cerebrospinal

Case	Condition immediately before treatment							Treatment. Gm. neocryl per km. body weight × no. of doses. Interval between doses
	Clinical	Body weight in lb.	Blood tryps.	Gland- juice tryps.	Protein %	Cells	Tryps.	
17	2 years. Malaise and headaches. Only slight somnolence. Glands in neck and axillae. Pulse 100.	126	—	+	0.020	4	—	0.045 × 10 weekly (Absent 2 weeks between 2nd and 3rd dose)
18	2 years. Severe headaches and slight somnolence. Glands in neck and axillae. Spleen just palpable. Pulse 84.	92	—	+	0.030	4	—	0.045 × 6 weekly (Absent 3 weeks between 1st and 2nd dose)
21	4 months. Generalized pains and headaches. Generalized adenitis. Pulse 120.	66	—	+	0.025	4	—	0.045 × 10 weekly
22	1 year. Occasional fever. Headaches, but not much somnolence. Numerous small glands in neck and axillae. Spleen palpable. Pulse 128.	112	—	+	0.030	4	—	0.060 × 10 weekly (Absent 2 weeks between 7th and 8th dose)
23	1 year. Headaches and somnolence, with occasional attacks of fever. Glands in neck and axillae. Spleen palpable 2". Pulse 124.	95	+	+	0.020	1	—	0.045 × 10 weekly

fluids), suffering from Gambian sleeping sickness, with neocryl

Condition after treatment

Time of examination	Clinical	Body weight in lb.	Blood tryps.	Gland-juice tryps.	C.S.F.			Remarks
					Protein %	Cells	Tryps.	
At 10th dose	Appears and feels quite well. No headaches, malaise or somnolence. Vision normal. Pulse 90. Urine n.a.c.	120	—	—	0.040	4	—	
8 weeks after 10th dose	Still apparently quite well. Pulse 84.	119	—	—				
16 weeks after 10th dose	Still quite well. Glands still palpable. Reflexes and vision normal. Pulse 78. Urine n.a.c.	124	—	—	0.025	2	—	
								Absconded after 6 doses. Then apparently quite well.
At 10th dose	Appears and feels quite well. No pains or headaches. Glands very small. Pulse 102. Vision normal.	72	—	—	0.030	4	—	
At 10th dose	Appears and feels quite well. No fever, headaches or somnolence. Glands too small to puncture. Spleen not palpable. Pulse 92. Reflexes and vision normal.	112	—	—	0.025	2	—	
At 10th dose	Appears and feels quite well. No headaches, fever or somnolence. Glands still present but spleen only just palpable. Pulse 104. Vision normal.	103	—	—	0.025	2	—	

TABLE I (Continued).—Showing the results of treating patients (with normal cerebrospinal

Case	Condition immediately before treatment							Treatment, Gm. neocryl per kgm. body weight × no. of doses. Interval between doses
	Clinical	Body weight in lb.	Blood tryps.	Gland- juice tryps.	Protein %	Cells	Tryps.	
27	1 month. Malaise, pain in neck, and headache. No somnolence. Large soft glands in neck and axillae. Pulse 96.	133	—	+	0.020	1	—	0.045 × 10 weekly
36	1 month. Headaches, but no somnolence. Very small glands in neck. Pulse 96.	124	+	+	0.035	6	—	0.045 × 3 weekly
44	1 year. Headaches and increasing somnolence. Generalized adenitis (small glands). Pulse 92.	148	—	+	0.030	4	—	0.045 × 10 weekly (Absent 2 weeks between 6th and 7th dose)
45	1 year. Generalized pains, but no definite headaches or somnolence. Small glands in neck and axillae. Pulse 84.	100	+	+	0.030	4	—	0.045 × 10 weekly (Absent 6 weeks between 1st and 2nd dose; 2 weeks between 6th and 7th dose)
47	2 months. Headaches. 1 month. Somnolence. Moderate-sized generalized adenitis. Pulse 130.	137	+	+	0.025	4	—	0.045 × 10 weekly (Absent 2 weeks between 1st and 2nd dose and 7th and 8th)
51	2 years. Abdominal pain, but no headaches or somnolence. 4 months. Amenorrhoea. Generalized adenitis. Spleen 4" palpable. Tenderness over gall-bladder. Pulse 146.	112	—	+	0.025	6	—	0.045 × 7 weekly

(fluids), suffering from Gambian sleeping sickness, with neocryl

Condition after treatment

Time of examination	Clinical	Body weight in lb.	Blood tryps.	Gland-juice tryps.	C.S.F.			Remarks
					Protein %	Cells	Tryps.	
At 10th dose	Appears and feels quite well. No pains or headache. Glands smaller. Vision normal. Pulse 84.	141	—	—	0.025	0	—	
5 weeks after 10th dose	Still quite fit. Pulse 96. Glands very small. Reflexes and vision normal. Urine n.a.c.	146	—	—	0.020	4	—	
At 3rd dose	Feeling better. No headaches.							Absconded after 3 doses.
At 10th dose	Appears and feels quite well. No headaches or somnolence. Glands very small. Pulse 84. Reflexes and vision normal.	150	—	—	0.030	5	—	
6 weeks after 1st dose	Reports absence was due to failure of vision, which is now improved. Vision and fundi now apparently normal.							Apparently definite temporary failure of vision after 1st dose.
At 10th dose	Appears and feels quite well. No pains. Glands very small. Pulse 84. Reflexes and vision normal.	115	—	—	0.030	0	—	
4 weeks after 10th dose	Still quite fit. Pulse 88. Vision normal.	113	—	—	0.030	2	—	
At 10th dose	Appears and feels quite well. No headaches or somnolence. Glands still present. Reflexes and vision normal. Pulse 84. Urine n.a.c.	136	—	—	0.025	7	—	
At 7th dose	Appeared clinically well. Menses commenced 3 weeks previously. Pulse 100.							Absconded after 7th dose.

TABLE I (Continued).—Showing the results of treating patients (with normal cerebrospinal

Case	Clinical	Condition immediately before treatment						Treatment: Gm. neocryl per kgm. body weight no. of doses, Interval between doses
		Body weight in lb.	Blood tryps.	Gland- juice tryps.	Protein %	C.S.F. Cells	Tryps.	
55	3 years. Malaise, headaches, somnolence, loss in weight and increasing weakness. Generalized adenitis. Spleen $1\frac{1}{2}$ " palpable. Pulse 108.	126	—	—	0.025	8	—	0.045 10 weekly (Absent 3 weeks between 6th and 7th doses)
57	1 year. Glands in neck. Headaches and recently somnolence. Glands in neck and axillae. Pulse 96.	60	—	—	0.025	1	—	0.045 10 weekly (Absent 2 weeks between 1st and 2nd dose; 7th and 8th; and 8th and 9th)
59	3 years. Headaches and some somnolence. Glands in neck and axillae. Spleen palpable. Pulse 96.	160	—	—	0.040	2	—	0.045 10 weekly
61	4 months. Loss in weight. Headaches and slight somnolence. Generalized adenitis. Spleen 1" palpable. Pulse 96.	105	—	—	0.020	5	—	0.045 6 weekly
62	6 months. Wasting, headaches and somnolence. Glands in neck and axillae. Pulse 126.	107	—	—	0.040	6	—	0.045 10 weekly
64	9 months. Occasional fevers, headaches. 3 months. Somnolence. Generalized adenitis. Pulse 120.	134	—	—	0.025	5	—	0.045 7 weekly (Absent 2 weeks between 1st and 2nd, and 6th and 7th doses)

fluids), suffering from Gambian sleeping sickness, with neocryl

Condition after treatment

Time of examination	Clinical	Body weight in lb.	Blood tryps.	Gland-juice tryps.	C.S.F.			Remarks
					Protein %	Cells	Tryps.	
At 10th dose	Appears quite well. No pains, headaches or somnolence. Glands smaller. Spleen slightly smaller (1" palp.). Reflexes and vision normal. Pulse 84. Urine n.a.c.	126	—	—	0.020	6	—	
At 10th dose	Appears and feels quite well. No headaches or somnolence. Glands small. Reflexes and vision normal. Pulse 86.	56	—	—	0.025	3	—	
4 weeks after 10th dose	Still quite well. Glands shrinking even smaller. Pulse 92. Vision normal.	60	—	—	0.025	3	—	
At 10th dose	Appears and feels quite well. No headaches or somnolence. Glands small. Spleen only just palpable. Reflexes and vision normal. Pulse 84.	158	—	—	0.040	1	—	
1 week after 1st dose	Complains of heaviness over eyes.							Absconded after 6th dose apparently well. Eye trouble so slight as to be quite indefinite.
1 week after 2nd dose	Eyes feel and appear normal. Headaches and somnolence disappeared.							
At 10th dose	Appears quite well. Glands small. Reflexes and vision normal. Pulse 92.	115	—	—	0.035	5	—	
								Absconded after 7th dose apparently well.

TABLE I (Continued).—Showing the results of treating patients (with normal cerebrospinal

Case	Condition immediately before treatment							Treatment. Gm. neocryl per kgm. body weight × no. of doses. Interval between doses
	Clinical	Body weight in lb.	Blood tryps.	Gland- juice tryps.	C.S.F.			
					Protein %	Cells	Tryps.	
67	2 months. Headaches. No somnolence. Small glands in neck. Pulse 144.	99	—	—	0.020	0	—	0.045 × 5 weekly
69	6 months. Generalized pain and headaches. 1 month. Somnolence. Glands in neck and axillae. Spleen 1" palpable. Marked lethargy. Pulse 104.	112	—	—	0.025	6	—	0.045 × 10 weekly
72	2 years. Pains over body. No headache, no somnolence. Generalized adenitis. Pulse 104.	128	—	—	0.025	5	—	0.045 × 10 weekly (Absent 2 weeks between 1st and 2nd, and 3rd and 4th doses)
73	8 years. Glands in neck. 3 months. Headaches, but no somnolence. Moderate and small glands in neck and axillae. Pulse 96.	125	—	—	0.030	6	—	0.045 × 1 26.2.37 (Did not return until 16.4.37)

(fluids), suffering from Gambian sleeping sickness, with neocryl

Condition after treatment								Remarks
Time of examination	Clinical	Body weight in lb.	Blood tryps.	Gland-juice tryps.	C.S.F.			
					Protein %	Cells	Tryps.	
								Absconded after 5th dose apparently well.
After 7th dose	Complained of slight abdominal pain.							Mild abdominal pain lasting a few days after 7th dose. Probably not toxic effect.
At 10th dose	Appears and feels quite well. No lethargy. No pains, headaches or somnolence. Glands moderate size. Spleen just palpable. Reflexes and vision normal. Pulse 86.	118	—	—	0.025	5	—	
At 10th dose	Appears and feels quite well. No pains, headaches or somnolence. Glands smaller. Reflexes and vision normal. Pulse 84.	123	—	—	0.020	3	—	
7 weeks after only dose	Feels quite well. No headaches. Pulse 120. Vision normal.	127	—	—	0.035	2	—	Owing to the long absence after 1st dose it was decided to watch events without further treatment. After 18 weeks, although no parasites were found, rapid pulse and loss in weight were noted, and Bayer 205 and tryparsamide were given.
13 weeks after only dose	Still apparently quite fit. Pulse 100.	125	—	—	0.030	4	—	
18 weeks after only dose	Appears well, but pulse 180 and some loss in weight. No headaches. Reflexes and vision normal.	109	—	—	0.030	3	—	

TABLE 1 (Continued).—Showing the results of treating patients (with normal cerebrospinal

Case	Condition immediately before treatment							Treatment. Gm. neocryl per kgm. body weight × no. of doses. Interval between doses
	Clinical	Body weight in lb.	Blood tryps.	Gland- juice tryps.	C.S.F.			
					Protein %	Cells	Tryps.	
79	1 year. Headaches and somno- lence. Generalized adenitis. Pulse 126.	112	—	—	0.035	5	—	0.045 × 10 weekly
80	1 year. Headaches and somno- lence. Glands in neck and axillae. Pulse 102.	56	—	—	0.025	2	—	0.045 × 10 weekly (Absent 2 weeks between 4th and 5th dose)
86	3 months. Headaches. 1 month. Somnolence. Genera- lized adenitis. Pulse 126.	60	—	—	0.030	6	—	0.045 × 10 weekly (Absent 5 weeks between 1st and 2nd dose)
88	6 months. Abdominal pain, head- aches and recently somnolence. Glands in neck and axillae. Pulse 134.	85	—	—	0.025	5	—	0.045 × 10 weekly
89	6 months. Abdominal pain and headaches, but no somnolence. Generalized adenitis. Pulse 96.	72	—	—	0.025	2	—	0.045 × 10 weekly
97	7 months. Generalized pain, head- aches and loss in weight, but no somnolence. Moderate to large glands in neck and axillae. Pulse 126.	81	—	—	0.025	4	—	0.045 × 10 weekly

fluids), suffering from Gambian sleeping sickness, with neocryl

Condition after treatment

Time of examination	Clinical	Body weight in lb.	Blood tryps.	Gland-juice tryps.	C.S.F.			Remarks
					Protein %	Cells	Tryps.	
At 10th dose	Appears and feels quite well. No headaches or somnolence. Glands small. Reflexes and vision normal. Pulse 120. Urine n.a.c.	128	—	—	0.030	0	—	
At 10th dose	Appears and feels quite well. No headaches or somnolence. Glands almost disappeared. Pulse 110. Reflexes and vision normal.	56	—	—	0.025	3	—	
6 weeks after 10th dose	Still quite well. Reflexes and vision normal. Pulse 120.	59	—	—	0.025	4	—	
At 10th dose	Appears and feels well. No headaches or somnolence. Glands smaller. Reflexes and vision normal. Pulse 90.	68	—	—	0.030	4	—	
2 weeks after 10th dose	Quite well. Pulse 90.	68						
At 10th dose	Feels quite well. No symptoms. Pulse 96. Reflexes and vision normal.	90	—	—	0.025	4	—	
At 10th dose	Appears and feels quite well. No pains or headaches. Pulse 78. Reflexes and vision normal.	78	—	—	0.025	5	—	
At 10th dose	Appears and feels quite well. No pains or headaches. Glands as originally. Pulse 96. Reflexes and vision normal.	87	—	—	0.020	4	—	

TABLE I (Continued).—Showing the results of treating patients (with normal cerebrospinal

Case	Condition immediately before treatment							Treatment. Gm. neocryl per km. body weight × no. of doses. Interval between doses
	Clinical	Body weight in lb.	Blood tryps.	Gland- juice tryps.	C.S.F.			
					Protein %	Cells	Tryps.	
99	4 months. Headaches and somnolence, with frequent fever. Dull facies. Generalized adenitis. Pulse 102.	110	—	+	0.030	2	—	0.045 × 10 weekly
101	1 year. Glands in neck. 1 month. Abdominal pain and somnolence. Generalized adenitis. Pulse 96.	82	—	+	0.035	5	—	0.045 × 10 weekly
102	1 year. Glands in neck. No headaches or somnolence. Generalized adenitis. Pulse 120.	153	—	+	0.025	5	—	0.045 × 1
104	2 months. Headaches and frequent fevers. No somnolence. Glands in neck and axillae. Spleen 3" palpable. Pulse 156.	77	—	+	0.020	2	—	0.045 × 5 weekly (Absent 6 weeks between 3rd and 4th doses)
105	1 month. Severe headache and fever. No somnolence. Generalized adenitis. Pulse 150.	90	—	+	0.030	2	—	0.045 × 5 weekly
109	6 months. Pain in back and headaches. Generalized adenitis. Spleen 6" palpable. Pulse 114.	126	—	+	0.040	5	—	0.045 × 10 weekly

fluids), suffering from Gambian sleeping sickness, with neocryl

Condition after treatment								
Time of examination	Clinical	Body weight in lb.	Blood tryps.	Gland-juice tryps.	C.S.F.			Remarks
					Protein %	Cells	Tryps.	
At 10th dose	Appears and feels quite well. No fever, headaches or somnolence. Glands very small. Pulse 84. Reflexes and vision normal.	111	—	—	<0.020	0	—	
At 10th dose	Appears and feels quite well. No pains or somnolence. Glands too small to puncture. Pulse 84. Reflexes and vision normal.	84	—	—	0.025	0	—	
								Absconded after 1st dose.
6 weeks after 3rd dose	Reports absence due to failure of vision for 10 days after 3rd dose. Vision now normal. Fundi normal. General condition improved. No headaches or somnolence.							After 3rd dose apparently definite failure of vision, which recovered. Absconded after 5 doses apparently well with normal vision.
								Absconded after 5th dose clinically much improved.
At 10th dose	Appears and feels quite well. No pains or headaches. Glands smaller. Spleen 4" palpable. Pulse 72. Reflexes and vision normal.	133	—	—	0.030	5	—	
5 weeks after 10th dose	Still quite well. Pulse 72. Reflexes and vision normal.	134	—	—	0.030	4	—	

TABLE I (Continued).—Showing the results of treating patients (with normal cerebrospinal

Case	Clinical	Condition immediately before treatment			C.S.F.			Treatment, Gm. neocryl per kgm. body weight no. of doses, Interval between doses
		Body weight in lb.	Blood tryps.	Gland- juice tryps.	Protein %	Cells	Tryps.	
111	Some years. Headaches, abdominal pains and somnolence. Generalized adenitis. Spleen 2" palpable. Pulse 126.	84	—	+	0.030	3	—	0.045 × 10 weekly (Absent 4 weeks between 1st and 2nd doses, and 2 weeks between 2nd and 3rd doses)
112	1 year. Headaches and lately somnolence. Glands in neck and axillae. Pulse 102.	116	—	+	0.030	4	—	0.045 × 10 weekly
116	3 weeks. Pains in body. No headaches or somnolence. Glands in left side neck. Pulse 126.	128	—	+	0.030	5	—	0.045 × 10 weekly (Absent 2 weeks between 2nd and 3rd doses)
118	3 months. Headaches, somnolence and loss in weight. Generalized adenitis. Pulse 132.	100	—	+	0.030	5	—	0.045 × 10 weekly
119	8 months. Loss in weight and headaches. 3 months. Somnolence, glands in neck and axillae. Pulse 132.	110	—	+	0.030	5	—	0.045 × 5 weekly
121	1 year. Glands in neck, loss in weight and occasional fever. 4 months. Headaches. 1 month. Somnolence and generalized adenitis. Pulse 114.	105	—	+	0.025	6	—	0.045 × 8 weekly (Absent 2 weeks between 2nd and 3rd doses)
126	2 years. Headaches. 6 months. Somnolence. Apathetic and dull. Generalized adenitis. Pulse 138.	119	—	+	0.030	2	—	0.045 × 10 weekly (Absent 3 weeks between 1st and 2nd doses)

fluids), suffering from Gambian sleeping sickness, with neocryl

Condition after treatment

Time of examination	Clinical	Body weight in lb.	Blood tryps.	Gland-juice tryps.	C.S.F.			Remarks
					Protein %	Cells	Tryps.	
At 10th dose	Appears and feels quite well. No pain, headaches or somnolence. Glands small. Spleen only just palpable. Pulse 90. Reflexes and vision normal.	92	—	—	0.025	5	—	
At 10th dose	Appears and feels quite well. No headaches or somnolence. Glands very small. Pulse 84. Reflexes and vision normal.	119	—	—	0.025	6	—	
At 10th dose	Appears and feels well. No headaches. Glands smaller. Pulse 90. Reflexes and vision normal.	130	—	—	0.040	6	—	
At 10th dose	Appears and feels quite well. No headaches or somnolence. Glands very small. Pulse 104. Reflexes and vision normal.	105	—	—	0.025	6	—	
								Absconded after 5th dose when apparently well.
								Absconded after 8th dose when apparently well.
At 10th dose	Appears and feels well. No headaches or somnolence. Lethargy gone. Glands small. Pulse 102. Reflexes and vision normal.	112	—	—	0.025	4	—	

TABLE II.—Showing the results of treating patients (with pathological cerebro-

Case	Condition immediately before treatment							Treatment. Gm. neocryl per kgm. body weight × no. of doses. Interval between doses
	Clinical	Body weight in lb.	Blood tryps.	Gland- juice tryps.	C.S.F.			
					Protein %	Cells	Tryps.	
4	6 months. Severe headaches and slight somnolence. Generalized body pains. Glands in neck and axillae. Spleen just palpable. Pulse 160.	150	—	—	0.030	10	—	0.045 × 10 weekly
6	4 months. Generalized pain and headaches. No somnolence. Small glands in neck and large soft glands in axillae. Spleen palpable. Pulse 100.	114	—	—	0.090	59	—	0.045 × 11 irregularly (Absent 6 weeks between 3rd and 4th doses; 2 weeks between 4th and 5th doses; 2 weeks between 5th and 6th doses; and 2 weeks between 8th and 9th doses)
9	6 months. Malaise and headaches. Slight somnolence. Glands in neck and axillae. Pulse 100.	147	—	+	0.210	1,100	++	0.06 × 11 weekly (Absent 2 weeks between 1st and 2nd doses)
10	3 months. Headaches and some somnolence. Glands in neck and axillae. Spleen 3" palpable. Pulse 124.	120	—	—	0.150	1,000	+	0.055 × 10 weekly

Condition after treatment								
Time of examination	Clinical	Body weight in lb.	Blood tryps.	Gland-juice tryps.	C.S.F.			Remarks
					Protein %	Cells	Tryps.	
At 10th dose	Appears and feels quite well. No pains, headaches or somnolence. Glands smaller. Spleen not palpable. Pulse 84. Reflexes and vision normal. Urine n.a.c.	157	—	—	0.025	60	—	Apparently definitely improved, except for increase in C. S. F. cell count.
1 week after 2nd dose	Better. No headaches. Vision normal.	120	—	—	0.050	40	—	Attended most irregularly, but was definitely improved, except for slight increase in C.S.F. cell count.
2 weeks after 5th dose	No headaches. No somnolence. Vision normal. Pulse 102.	126	—	—	0.050	125	—	
2 weeks after 8th dose	Still feeling well.		—	—	0.050	80	—	
2 weeks after 11th dose	Appears well. No headaches or somnolence. Glands very small in neck and moderate in axillae. Spleen palpable. Pulse 84. Reflexes and vision normal.	135	—	—	0.045	80	—	
At 11th dose	Appears and feels quite well. Leading normal life. No headaches or somnolence. Glands very small. Pulse 84. Reflexes and vision normal.	176	—	—	0.180	17	—	
5 weeks after 11th dose	(Said to have been seen 10 days ago, apparently perfectly fit.)							
At 10th dose	Appears and feels well. No headaches or somnolence. Glands very small. Spleen just palpable. Pulse 84. Reflexes and vision normal.	126	—	—	0.035	22	—	Impossible to say what was the sudden cerebral condition which apparently caused death, but in view of the changes in the C.S.F. 9 weeks after ceasing treatment it would be unwise to exclude a relapse, although parasites were not found.
9 weeks after 10th dose	Still appears quite well. Pulse 84.	130	—	—	0.100	250	—	
11 weeks after 10th dose	Reported that he became suddenly ill 7 days ago, complaining of severe headache, and that he died 4 days ago.							

TABLE II (Continued).—Showing the results of treating patients (with pathological cerebrospinal

Case	Condition immediately before treatment							Treatment. Gm. neocryl per kgm. body weight × no. of doses. Interval between doses
	Clinical	Body weight in lb.	Blood tryps.	Gland- juice tryps.	Protein %	Cells	Tryps.	
11	3 months. Generalized pains, headaches and somnolence. Helpless and carried in. Small glands in neck and axillae. Spleen just palpable. Pulse 120.	88	—	+	0.150	300	+	0.055 × 10 weekly (Absent 2 weeks between 1st and 2nd doses) Followed 7 weeks later by second course of 0.055 × 3 weekly
13	2 years. Headaches and somnolence. Completely demented. Glands in neck and axillae. Pulse 120.	112	—	+	0.280	480	+	0.06 10 weekly
16	2 years. Generalized pains, loss in weight, headaches and somnolence. Glands in neck and axillae. Spleen palpable. Pulse 100.	112	—	+	0.060	55	+	0.045 × 10 weekly

Ruidi, suffering from Gambian sleeping sickness, with neocryl

Condition after treatment

Time of examination	Clinical	Body weight in lb.	Blood tryps.	Gland-juice tryps.	C.S.F.			Remarks
					Protein %	Cells	Tryps.	
At 10th dose	Apparently quite well. Walks normally. Glands too small to puncture. Pulse 76. Spleen just palpable.	98	—	—	0.035	15	—	Relapsed 6 weeks after finishing treatment. Given further course of 0.055 gm. neocryl per kgm. body weight weekly, and was so improved after 3 doses that she absconded from hospital and was not seen again.
7 weeks after 10th dose	Complaining of headaches for 6 days and vomiting for 3 days. Lethargic. No evidence of localized nerve lesion. Glands still small. Spleen 2" palpable. Pulse 104.	90	—	—	0.240	600	—	
At 10th dose	Appears and feels quite well. Mentally normal and leading normal life. No headaches or somnolence. Glands too small to puncture. Pulse 96. Reflexes and vision normal.	115	—	—	0.070	40	—	Remarkable improvement under treatment, but occasional headaches, some somnolence, and condition of C. S. F. 15 weeks after finishing treatment suggest relapse, although no parasites were found.
15 weeks after 10th dose	Appears physically and mentally fairly well, but occasionally gets slight headaches, has tremor and occasional periods of slight somnolence. Pulse 104. Glands small. Reflexes and vision normal.	119	—	—	0.090	130	—	
1 week after 2nd dose	Feels better; no somnolence, but still gets slight headache.	—	—	—	0.030	40	—	
At 10th dose	Appears and feels quite well. No pains, headaches or somnolence. Glands and spleen as originally. Pulse 96. Reflexes and vision normal.	117	—	—	0.035	6	—	

TABLE II (Continued).—Showing the results of treating patients (with pathological cerebrospinal

Case	Condition immediately before treatment							Treatment. Gm. neocryl per kgm. body weight × no. of doses. Interval between doses
	Clinical	Body weight in lb.	Blood tryps.	Gland- juice tryps.	C.S.F.			
					Protein %	Cells	Tryps.	
19	1 month. Headaches and weak- ness. No somnolence. General- ized adenitis. Pulse 104.	114	+	+	0.060	10	—	0.045 6 weekly
20	1 year. Generalized pains, head- aches and somnolence. Cere- bration and movements very slow. Glands in neck and axillae.	113	—	+	0.125	650	+	0.045 6 weekly
24	2 years. Headaches and recently somnolence. Glands in neck and axillae. Pulse 94.	150	—	+	0.050	550	+	0.045 9 weekly (Absent 2 weeks between 1st and 2nd doses; and 3 weeks between 8th and 9th) Followed immedi- ately by further course of 0.075 × 1; 0.06 3 weekly

fluids), suffering from Gambian sleeping sickness, with neocryl

Condition after treatment								Remarks
Time of examination	Clinical	Body weight in lb.	Blood tryps.	Gland-juice tryps.	C.S.F.			
					Protein %	Cells	Tryps.	
								Absconded after 6th dose apparently well.
1 week after 1st dose			—	—	0.080	850	—	Absconded after 6th dose apparently improved. Complained of dizziness 5 weeks after commencing treatment, but stated a week later that this had passed off.
At 9th dose	Feels quite well. No headaches or somnolence. Glands very small. Pulse 76. Reflexes and vision normal.	147	—	—	0.050	200	+	Relapsed during treatment, with tryps. in C.S.F. Further course with larger dosage started (vomited after 1st dose and had abdominal pain, therefore dose slightly reduced). Clinically well and no parasites seen after 4 doses of 2nd course, but C. S. F. still abnormal 4 weeks later.
4 weeks after 4th dose of further course	Appears and feels well. No headaches or somnolence. Glands small. Pulse 72. Reflexes and vision normal.	153	—	—	0.050	180	—	
7 weeks after 4th dose of further course	Still feels quite well.							

TABLE II (*Continued*).—Showing the results of treating patients (with pathological cerebrospinal fluid)

Case	Condition immediately before treatment							Treatment. Gm. neocryl per kgm. body weight × no. of doses. Interval between doses
	Clinical	Body weight in lb.	Blood tryps.	Gland- juice tryps.	Protein %	C.S.F. Cells	Tryps.	
25	1 year. Generalized pains and headaches. 3 months. Somnolence. Incontinence of faeces and urine. Glands in neck and axillae. Can only walk with assistance. Pulse 124.	107	—	+	0.070	330	+	0.045 × 10 weeks
26	5 years. Malaise, occasional fever and headaches. 4 months. Somnolence. Incontinence of urine and faeces. Glands in neck and axillae. Pulse 88.	136	—	+	0.040	175	+	0.045 × 2 weekly
28	1 year. Headaches and somnolence. Glands in neck and axillae. Pulse 112.	112	+	+	0.090	200	+	0.045 × 1
29	4 months. Generalized pains and headaches, but no somnolence. Small glands in neck and axillae. Pulse 104.	135	—	+	0.030	60	+	0.045 × 1
30	9 months. Generalized pains, headaches and slight somnolence. Glands in neck and axillae. Pulse 120.	130	—	+	0.120	180	+	0.045 × 1

fluids), suffering from Gambian sleeping sickness, with neocryl

Condition after treatment								Remarks
Time of examination	Clinical	Body weight in lb.	Blood tryps.	Gland-juice tryps.	C.S.F.			
					Protein %	Cells	Tryps.	
1 week after 1st dose	Seems improved. Somnolence less. Walking improved. No longer incontinent. Vision normal.			—	0.070	100		Early in course this patient was moved to Georgetown, and a supply of drug was sent there for treatment to be continued.
At 10th dose	Reported "Clinically much better."							
8 weeks after 10th dose	C.S.F. sent by steamer. Examined 39 hours after lumbar puncture.				0.060	75		
12 weeks after 10th dose	Reported that "Glands in neck present but small and hard. Some somnolence by day. Weight decreasing. Condition disimproving."							
1 week after 1st dose	Somewhat improved. No longer incontinent. Vision normal.		—	—	0.050	300	—	Absconded after 2nd dose.
								Absconded after 1st dose.
								Absconded after 1st dose.
24 hours after 1st dose				—				Absconded after 1st dose.

TABLE II (Continued).—Showing the results of treating patients (with pathological cerebrospinal

Case	Condition immediately before treatment							Treatment. Gm. neocryl per kgm. body weight × no. of doses. Interval between doses
	Clinical	Body weight in lb.	Blood tryps.	Gland- juice tryps.	Protein %	C.S.F. Cells	Tryps.	
31	9 months. Headaches and somnolence. Incontinence of urine and faeces. Generalized adenitis. Pulse 180. Walks with difficulty.	104	—	+	0.120	680	+	0.045 × 10 weekly
32	3 months. Headaches and somnolence. Glands in neck and axillae. Pulse 100.	90	+	+	0.030	104	+	0.045 × 7 weekly (Absent 4 weeks between 6th and 7th doses)
33	3 months. Headaches and some somnolence. Generalized adenitis. Pulse 160.	80	—	+	0.100	300	+	0.045 × 7 weekly (Absent 2 weeks between 5th and 6th doses)
34	6 months. Loss in weight, pains in head and some somnolence. Glands in neck and axillae. Pulse 100.	120	—	+	0.040	28	+	0.045 × 8 weekly (Absent 3 weeks between 4th and 5th doses)
35	3 years. Generalized pains, headaches and somnolence. Glands in neck and axillae.	130	—	+	0.060	100	+	0.045 × 8 weekly
37	3 years. Headaches. 1 year. Somnolence. Deeply lethargic; unable to walk. Glands in neck and axillae. Pulse 94.	121	—	+	0.060	40	+	0.045 × 10 weekly

fluids), suffering from Gambian sleeping sickness, with neocryl

Condition after treatment								Remarks
Time of examination	Clinical	Body weight in lb.	Blood tryps.	Gland-juice tryps.	C.S.F.			
					Protein 0.0	Cells	Tryps.	
At 10th dose	Reported "Clinically improved."							After 1st dose was sent to Georgetown to complete treatment, but no examinations of fluid, etc., were carried out there at end of course.
11 weeks after 10th dose	Reported to be "Well again and farming."							
								Absconded after 7th dose apparently well.
								Had some days' abdominal pain and vomiting after 1st dose, which then passed off. Absconded after 7th dose apparently well.
3 weeks after 4th dose	Feels quite well. Glands very small. Pulse 72. Vision normal.	120	—	—	0.040	41	—	Absconded after 8th dose clinically well.
								Absconded after 8th dose apparently well.
At 10th dose	Much improved. Mentally bright and walks normally. No headaches or somnolence. Glands small. Pulse 120. Reflexes and vision normal.	121	—	—	0.060	5	—	

TABLE II (Continued).—Showing the results of treating patients (with pathological cerebrospinal

Case	Condition immediately before treatment							Treatment. Gm. neocryl per km. body weight × no. of doses. Interval between doses
	Clinical	Body weight in lb.	Blood tryps.	Gland- juice tryps.	Protein %	Cells	Tryps.	
38	7 months. Loss in weight, general- ized pains and headaches. 1 month. Somnolence. Fixed and dull facies. Small glands in neck and axillae. Pulse 132. Vertical nystagmus but no fur- ther evidence of localized nerve lesion.	126	—	+	0.090	800	+	0.045 × 9 weekly Followed immedi- ately by further course of 0.09 5 weekly
39	2 years. Headaches and loss in weight. 2 months. Somnolence. Deeply somnolent with obvious severe headache when roused. Glands in neck and axillae. Pulse 84.	144	—	+	0.130	500	+	0.045 × 10 weekly
40	1 year. Headaches, loss in weight and somnolence. Small glands in neck and axillae. Pulse 134.	82	—	+	0.050	125	+	0.045 × 10 weekly (Absent 2 weeks between 7th and 8th doses; and 2 weeks between 8th and 9th doses)
41	1 year. Headaches and somno- lence. Glands in neck and axillae. Pulse 128.	112	—	+	0.040	32	+	0.045 × 10 weekly (Absent 2 weeks between 2nd and 3rd doses; 2 weeks between 6th 7th; and 2 weeks between 7th and 8th doses)

fluids), suffering from Gambian sleeping sickness, with neocryl

Condition after treatment								
Time of examination	Clinical	Body weight in lb.	Blood tryps.	Gland-juice tryps.	C.S.F.			Remarks
					Protein %	Cells	Tryps.	
1 week after 9th dose	Feels quite well. Facies and mentality apparently normal. No headaches or somnolence. Glands very small. Pulse 72. Nystagmus not present. Reflexes and vision normal.	132	—	—	0.040	110	+	Relapsed during treatment with tryps. in C.S.F. Further course of large doses given without any signs of toxic effects. 9 weeks after finishing this further course there was some loss in weight, and protein and cell content of C. S. F. were worse, although tryps. could not be found.
1 week after 5th dose of further course	Feels well. Pulse 84.	136	—	—	0.040	80	—	
9 weeks after 5th dose of further course	Appears well, but walks slowly. No headaches or somnolence. Glands small. Pulse 84. Reflexes and vision normal. No nystagmus.	127	—	—	0.090	240	—	
At 10th dose	Appears and feels quite well. No headaches or somnolence. Glands still present. Pulse 76. Reflexes and vision normal.	140	—	—	0.045	28	—	
At 10th dose	Appears and feels quite well. No headaches or somnolence. Glands too small to puncture. Pulse 120. Reflexes and vision normal.	98	—	—	0.045	110	—	
At 10th dose	Appears and feels much improved, but still gets occasional headaches. No somnolence. Glands smaller. Pulse 78. Reflexes and vision normal.	114	—	—	0.030	7	—	Occasional headaches remained after treatment, but no definite cause could be found for this.
6 weeks after 10th dose	Still quite well but for occasional headache. Pulse 108.	117	—	—	0.030	5	—	

TABLE II (Continued).—Showing the results of treating patients (with pathological cerebrospinal

Case	Condition immediately before treatment							Treatment. Gm. neocryl per kgm. body weight × no. of doses. Interval between doses
	Clinical	Body weight in lb.	Blood tryps.	Gland- juice tryps.	Protein %	C.S.F. Cells	Tryps.	
42	3 years. Headaches and somnolence. Deeply lethargic. Glands in neck and axillae. Pulse 90.	140	—	+	0.120	400	+	0.045 × 10 weekly (Absent 2 weeks between 4th and 5th doses; 2 weeks between 8th and 9th; and 2 weeks between 9th and 10th doses)
43	1 year. Generalized pains and headaches. 3 months. Somnolence. Helpless and carried in. Glands in neck and axillae. Pulse 108.	108	—	+	0.090	400	+	0.045 × 10 weekly
46	1 year. Headaches. 2 months. Somnolence. Marked lethargy and dullness; difficulty in walking; oedema of eyes. Generalized adenitis. Pulse 100.	143	—	+	0.100	2,000	+	0.045 × 8 weekly (Absent 2 weeks between 2nd and 3rd doses)
48	4 months. Headaches and somnolence. Emaciated, weak, deeply lethargic and hardly able to stand. Small glands in neck and axillae. Pulse 136.	90	—	+	0.040	35	+	0.045 × 10 weekly

fluids), suffering from Gambian sleeping sickness, with neocryl

Condition after treatment								Remarks
Time of examination	Clinical	Body weight in lb.	Blood tryps.	Gland-juice tryps.	C.S.F.			
					Protein %	Cells	Tryps.	
At 10th dose	Appears and feels quite well. No headaches or somnolence. Glands smaller. Pulse 84. Reflexes and vision normal. (Disappeared before lumbar puncture was done.)	145	—	—				
At 10th dose	Appears and feels well. Walks well. Glands very small. Pulse 100. Reflexes and vision normal.	114	—	—	0.050	32	—	
3 weeks after 10th dose	Still well. Walks normally. Pulse 100.	119						
48 hours after 1st dose			—	—			—	Absconded after 8th dose apparently well.
3½ hours after 1st dose			—	—				
6 hours after 1st dose			—	—	0.080	100	+	
24 hours after 1st dose			—	—	0.080	175	+	
1 week after 1st dose	Feeling improved. Headaches less, but still somnolent. Able to stand. Vision normal.		—	—	0.120	200	—	
At 10th dose	Appears much better and feels well. Walks quite well. No headaches or somnolence. Glands small. Pulse 100. Reflexes and vision normal.	107	—	—	0.080	48	—	

TABLE II (Continued).—Showing the results of treating patients (with pathological cerebrospinal

Case	Condition immediately before treatment							Treatment. Gm. neocryl per kgm. body weight × no. of doses. Interval between doses
	Clinical	Body weight in lb.	Blood tryps.	Gland- juice tryps.	C.S.F.			
					Protein %	Cells	Tryps.	
49	5 years. Glands in neck. 2 months. Headaches and somno- lence. Lethargic, with dull facies. Generalized adenitis. Pulse 92.	60	—	+	0.100	600	+	0.045 × 5 weekly
50	4 months. Generalized pains. Loss in weight, headaches and increasing somnolence. Glands in neck and axillae. Pulse 76.	138	—	+	0.040	21	—	0.045 × 8 weekly (Absent 4 weeks between 6th and 7th doses; and 6 weeks between 7th and 8th doses)
52	1 year. Generalized pains, head- aches and increasing somno- lence. Generalized adenitis. Pulse 72.	112	—	+	0.045	250	+	0.045 × 8 weekly
53	6 months. Glands in neck, head- aches and somnolence. Lethar- gic, with dull facies; oedema of face. Generalized adenitis. Pulse 84.	94	—	+	0.040	30	—	0.045 × 9 weekly (Absent 2 weeks between 1st and 2nd doses)
54	1 year. Generalized pains, head- aches and recently somnolence. Generalized adenitis. Pulse 150.	104	—	+	0.035	30	+	0.045 × 10 weekly (Absent 2 weeks between 1st and 2nd doses; 2 weeks between 5th and 6th; and 2 weeks between 9th and 10th doses)

fluids), suffering from Gambian sleeping sickness, with neocryl

Condition after treatment								Remarks
Time of examination	Clinical	Body weight in lb.	Blood tryps.	Gland-juice tryps.	C.S.F.			
					Protein %	Cells	Tryps.	
3 hours after 1st dose 1 week after 1st dose				— —				Absconded after 5th dose apparently well.
At 8th dose	Appears and feels quite well. No headaches or somnolence. Glands very small. Pulse 72. Reflexes and vision normal.	142	—	—	0.030	4	—	Absconded after 8th dose apparently well.
								Absconded after 8th dose apparently well.
								Absconded after 9th dose apparently well.
At 10th dose	Appears and feels well. No pains, headaches or somnolence. Glands smaller. Pulse 84. Reflexes and vision normal.	114	—	—	0.030	10	—	

TABLE II (Continued).—Showing the results of treating patients (with pathological cerebrospinal

Case	Condition immediately before treatment							Treatment. Gm. neocryl per km. body weight × no. of doses. Interval between doses
	Clinical	Body weight in lb.	Blood tryps.	Gland- juice tryps.	Protein %	C.S.F. Cells	Tryps.	
56	1 year. Generalized pains, loss in weight, headaches and somnolence. Deeply lethargic and carried in. Typical facies. Generalized adenitis. Pulse 110.	70	—	—	0.090	90	—	0.045 × 10 weekly (Absent 2 weeks between 1st and 2nd doses; and 8 weeks between 3rd and 4th doses)
58	1 year. Glands in neck. Headaches, and recently deep somnolence. Generalized adenitis. Pulse 100.	50	—	+	0.070	300	+	0.045 × 10 weekly
60	8 months. Abdominal pain and loss in weight. 2 months. Headaches and somnolence. Glands in neck and axillae. Spleen 2" palpable. Pulse 120.	100	—	+	0.040	175	+	0.045 × 11 weekly
63	5 years. Gradual loss of health. 4 months. Headaches. 3 months. Somnolence. Generalized adenitis. Pulse 102.	114	—	+	0.035	22	—	0.045 × 3 weekly

fluids), suffering from Gambian sleeping sickness with neocryl

Condition after treatment								Remarks
Time of examination	Clinical	Body weight in lb.	Blood tryps.	Gland-juice tryps.	C.S.F.			
					Protein %	Cells	Tryps.	
8 weeks after 3rd dose	Feels well. Walks normally. Vision normal.	76	—	—	0.090	70	—	Although helpless at first, was sufficiently improved after 3 doses to be persuaded only with difficulty to return for further treatment. Hence the long interval between 3rd and 4th doses.
At 10th dose	Appears and feels well. Lethargy definitely less. No pains, headaches or somnolence. Glands very small. Pulse 102. Reflexes and vision normal.	84	—	—	0.030	90	—	
At 10th dose	Appears and feels quite well. No headaches or somnolence. Glands small. Pulse 120. Reflexes and vision normal.	54	—	—	0.080	250	—	
At 11th dose	Appears and feels quite well. No pains, headaches or somnolence. Glands very small. Spleen only just palpable. Pulse 80. Reflexes and vision normal.	106	—	—	0.025	66	—	Relapsed 8 weeks after end of course of treatment.
10 weeks after 11th dose	Still feels fairly well, but for 2 weeks has had occasional headaches. No somnolence. Glands too small to puncture. Pulse 78. Reflexes and vision normal.	110	—	—	0.065	570	—	
								Absconded after 3rd dose apparently much improved, headache and somnolence having disappeared.

TABLE II (Continued).—Showing the results of treating patients (with pathological cerebrospinal

Case	Condition immediately before treatment							Treatment. Gm. neocryl per kgm. body weight × no. of doses. Interval between doses
	Clinical	Body weight in lb.	Blood tryps.	Gland- juice tryps.	Protein %	Cells	Tryps.	
65	6 months. Loss in weight, occasional fever, headaches and some somnolence. Glands in neck and axillae. Pulse 102.	111	—	+	0.035	44	—	0.045 × 10 weekly
66	9 months. Loss in weight, pains in limbs and headaches. 3 months. Somnolence. Generalized adenitis. Pulse 78.	134	—	+	0.140	750	+	0.045 × 10 weekly
68	9 months. Generalized pains and headaches. 4 months. Somnolence. Generalized adenitis. Pulse 108.	144	—	+	0.090	165	—	0.045 × 2 weekly
70	4 years. Generalized pains and headaches. 4 months. Somnolence. Deeply lethargic and in poor condition, with sores on skin. Generalized adenitis. Pulse 114.	153	—	+	0.065	535	—	0.045 × 3 weekly (Absent 3 weeks between 1st and 2nd doses)
71	6 months. Headaches. 2 months. Somnolence. Dull facies. Glands in neck and axillae. Pulse 96.	124	—	+	0.055	80	+	0.045 × 3 weekly (Absent 2 weeks between 2nd and 3rd doses)
74	6 months. Abdominal pain, loss in weight and headaches. 4 months. Somnolence. Deeply lethargic. Generalized adenitis. Pulse 90.	113	—	+	0.060	175	+	0.045 × 4 weekly

Condition after treatment								Remarks
Time of examination	Clinical	Body weight in lb.	Blood tryps.	Gland-juice tryps.	C.S.F.			
					Protein %	Cells	Tryps.	
At 10th dose	Appears and feels quite well. No fever, headaches or somnolence. Glands very small. Pulse 76. Reflexes and vision normal.	118	—	—	0.035	6	—	
At 10th dose	Appears and feels well. No pains, headaches or somnolence. Glands smaller. Pulse 90. Reflexes and vision normal.	152	—	—	0.045	13	—	
								Absconded after 2nd dose somewhat better.
								Absconded after 3rd dose somewhat improved.
								Absconded after 3rd dose improved.
1 week after 1st dose	Still lethargic, but headache is less. Complains of dullness of vision and pains in eyes. Some apparent loss of colour-perception. Fundi normal in appearance.							Some slight diminution in vision after 1st dose, which passed off without interruption of treatment. Absconded after 4 doses, improved and with apparently normal vision.
1 week after 2nd dose	Vision now normal.							
1 week after 3rd dose	Vision normal. General improvement.							

TABLE II (Continued).—Showing the results of treating patients (with pathological cerebrospinal

Case	Condition immediately before treatment							Treatment. Gm. neocryl per kgm. body weight × no. of doses. Interval between doses
	Clinical	Body weight in lb.	Blood tryps.	Gland- juice tryps.	C.S.F.			
					Protein %	Cells	Tryps.	
75	2 months. Loss in weight and headaches. No somnolence. Dull facies. Glands in neck and axillae. Pulse 84.	138	—	—	0.040	22	—	0.045 × 4 weekly
76	2 months. Headaches and somnolence. Glands in neck and axillae. Pulse 96.	64	—	—	0.040	60	—	0.045 × 10 weekly
77	4 months. Losing weight. 2 months. Headaches and somnolence. Generalized adenitis. Pulse 108.	119	—	—	0.035	15	—	0.045 × 10 weekly (Absent 2 weeks between 2nd and 3rd doses; and 5 weeks between 5th and 6th doses)
78	9 months. General ill health. Dull facies. Glands in neck and axillae. Pulse 138.	130	—	+	0.210	800	—	0.045 × 4 weekly
81	1 year. Headaches. 1 month. Somnolence. Glands in neck and axillae. Spleen 2" palpable. Pulse 102.	75	—	—	0.035	30	—	0.045 × 10 weekly (Absent 2 weeks between 4th and 5th doses, and between 8th and 9th doses)
82	3 years. Headaches. 9 months. Somnolence. Generalized adenitis. Pulse 156.	70	—	+	0.120	540	+	0.045 × 7 weekly (Absent 6 weeks between 4th and 5th doses)

fluids), suffering from Gambian sleeping sickness, with neocryl

Condition after treatment								Remarks
Time of examination	Clinical	Body weight in lb.	Blood tryps.	Gland-juice tryps.	C.S.F.			
					Protein %	Cells	Tryps.	
								Absconded after 4th dose.
At 10th dose	Appears and feels well. No headaches or somnolence. Glands very small. Pulse 100. Reflexes and vision normal.	65	—	—	0.030	22	—	
5 weeks after 5th dose	Seems quite well. No headaches or somnolence. Glands small. Pulse 108. Reflexes and vision normal.	123	—	—	0.045	100	—	
At 10th dose	Appears and feels well. No headaches or somnolence. Glands small. Pulse 90. Reflexes and vision normal.	128	—	—	0.030	44	—	
								Absconded after 4th dose apparently improved.
At 10th dose	Appears and feels well. No headaches or somnolence. Glands smaller. Spleen just palpable. Pulse 100. Reflexes and vision normal.	77	—	—	0.030	10	—	
								Absconded after 7th dose much improved.

TABLE II (Continued).—Showing the results of treating patients (with pathological cerebrospinal

Case	Condition immediately before treatment							Treatment. Gm. neocryl per kgm. body weight × no. of doses. Interval between doses
	Clinical	Body weight in lb.	Blood tryps.	Gland- juice tryps.	Protein %	Cells	Tryps.	
83	1 year. Generalized pains, occasional fever and frequent headaches. 2 months. Somnolence. Glands in neck and axillae. Spleen 2" palpable. Pulse 150.	98	—	+	0.090	300	+	0.045 × 10 weekly (Absent 2 weeks between 4th and 5th doses)
84	1 year. Headaches and somnolence. Generalized adenitis. Spleen 2" palpable. Pulse 90.	126	—	+	0.120	315	+	0.045 × 8 weekly (Absent 2 weeks between 3rd and 4th doses; 8 weeks between 4th and 5th doses; and 3 weeks be- tween 6th and 7th doses)
85	2 years. Generalized pain and headaches. 6 months. Somnolence. Glands in neck and axillae. Pulse 120.	112	—	+	0.045	200	+	0.045 × 10 weekly
87	9 months. Headaches and somnolence. Deeply lethargic. Generalized adenitis. Pulse 116.	129	—	+	0.090	125	+	0.045 × 1

(fluids), suffering from Gambian sleeping sickness, with neocryl

Condition after treatment								Remarks
Time of examination	Clinical	Body weight in lb.	Blood tryps.	Gland-juice tryps.	C.S.F.			
					Protein %	Cells	Tryps.	
At 10th dose	Appears quite well. No headaches or somnolence. Glands small. Spleen just palpable. Pulse 84. Reflexes and vision normal.	105	—	—	0.030	20	—	
8 weeks after 4th dose	Says long absence was due to failure of vision which is now recovered. Vision and fundi normal. No headaches or somnolence. Reflexes normal. Glands small.	130	—	—	0.090	65	—	Definite temporary optic damage. Pains in neck and protein and cell content of C.S.F. after 8 irregular doses suggest relapse although no tryps. were found. Absconded after 8th dose.
At 8th dose	Well but for slight pain in neck. No headaches or somnolence. Pulse 90. Reflexes and vision normal.		—	—	0.140	350	—	
At 10th dose	Appears and feels well. No headaches or somnolence. Glands too small to puncture. Pulse 78. Reflexes and vision normal.	118	—		0.030	25	—	
16 weeks after 1st dose	Not now lethargic. No headaches or somnolence. Pulse 96. Glands as before. Reflexes and vision normal.	138	—	+	0.090	86	+	Absconded for 16 weeks after 1st dose. Then returned because glands were still present and because of general malaise. Then given 0.5 gm. of Bayer 205 and routine course of try-parsamide.

TABLE II (Continued).—Showing the results of treating patients (with pathological cerebrospinal

Case	Condition immediately before treatment							Treatment. Gm. neocryl per km. body weight × no. of doses. Interval between doses
	Clinical	Body weight in lb.	Blood tryps.	Gland- juice tryps.	C.S.F.			
					Protein %	Cells	Tryps.	
90	9 months. Headaches and somno- lence. Generalized adenitis. Pulse 120.	106	—	+	0.100	610	+	0.045 × 10 weekly (Absent 2 weeks between 3rd and 4th doses)
91	5 months. Generalized pains and malaise. Glands in neck and axillae. Spleen 3" palpable. Pulse 114.	110	—	+	0.180	300	—	0.045 × 10 weekly (Absent 2 weeks between 3rd and 4th doses)
92	3 months. Headaches and somno- lence. Marked lethargy and typical facies. Generalized adenitis. Pulse 120.	139	—	+	0.135	1,600	+	0.045 × 7 weekly
93	2 years. Malaise. 3 months. Loss of weight. Head- aches, but no somnolence. Gen- eralized adenitis. Pulse 108.	148	—	+	0.090	96	+	0.045 × 6 weekly
94	3 months. Headaches and fre- quent fevers. 2 months. Somnolence. Glands in neck and axillae. Pulse 96.	60	—	+	0.030	14	—	0.045 × 10 weekly
95	5 months. Generalized pains and headaches. 2 months. Somnolence. Glands in neck and axillae. Pulse 132.	135	—	+	0.030	20	+	0.045 × 6 weekly (Absent 2 weeks between 5th and 6th doses)

(fluids), suffering from Gambian sleeping sickness, with neocryl

Condition after treatment								Remarks
Time of examination	Clinical	Body weight in lb.	Blood tryps.	Gland-juice tryps.	C.S.F.			
					Protein %	Cells	Tryps.	
At 10th dose	Appears well. Glands smaller. Pulse 72. Reflexes and vision normal.	113	—	—	0.090	50	—	
5 weeks after 10th dose	Still quite well. Glands almost disappeared. Pulse 90. Reflexes and vision normal.	115	—	—	0.070	30	—	
At 10th dose	Appears and feels well. Glands small. Spleen just palpable. Pulse 96. Reflexes and vision normal.	120	—	—	0.070	10	—	
5 weeks after 10th dose	Still quite fit. Glands small. Pulse 90. Reflexes and vision normal.	125	—	—	0.045	10	—	
								Absconded after 7th dose very much improved.
								Absconded after 6th dose much improved.
At 10th dose	Appears and feels well. No headache or somnolence. Glands smaller. Pulse 96. Reflexes and vision normal.	64	—	—	<0.020	5	—	
								Absconded after 6th dose improved.

TABLE II (Continued).—Showing the results of treating patients (with pathological cerebrospinal

Case	Condition immediately before treatment							Treatment. Gm. neocryl per kgm. body weight × no. of doses. Interval between doses
	Clinical	Body weight in lb.	Blood tryps.	Gland- juice tryps.	C.S.F.			
					Protein %	Cells	Tryps.	
96	4 months. Malaise, loss in weight and headaches. 2 months. Somnolence. Generalized adenitis. Pulse 102.	122	—	+	0.060	240	+	0.045 × 10 weekly
98	6 months. Amenorrhoea. 3 months. Loss in weight and severe headaches. No somnolence. Generalized adenitis. Pulse 96.	105	—	+	0.300	400	+	0.045 × 10 weekly (Absent 3 weeks between 9th and 10th doses)
100	1 year. Glands in neck. 2 months. Headaches and somnolence. Generalized adenitis. Spleen 1" palpable. Pulse 150.	126	—	+	0.050	170	+	0.045 × 5 weekly
103	2 years. Headaches and somnolence, with frequent fevers. Generalized adenitis. Pulse 120.	65	—	+	0.025	18	—	0.045 × 1 weekly
106	1 year. Headaches. Generalized adenitis. Pulse 90.	116	—	+	0.085	75	+	0.045 × 10 weekly (Absent 2 weeks between 3rd and 4th doses)

fluids), suffering from Gambian sleeping sickness, with neocryl

Condition after treatment								Remarks
Time of examination	Clinical	Body weight in lb.	Blood tryps.	Gland-juice tryps.	C.S.F.			
					Protein %	Cells	Tryps.	
At 10th dose	Appears and feels well. No pains, headaches or somnolence. Glands smaller. Pulse 102. Reflexes and vision normal.	138	—	—	0.045	58	—	
4 weeks after 10th dose	Still quite well. Glands even smaller. Pulse 84. Reflexes and vision normal.	136	—	—	0.035	90	—	
At 10th dose	Still has headaches and some somnolence. Recently started talking a lot of nonsense and is mentally deranged. Vision also very poor, cornea hazy and fundus only seen indistinctly. Still has amenorrhoea. Pulse 84. Tremor in hands.	121	—	—	0.070	94	—	Has gained weight and C. S. F. has improved, but clinically not improved and has become mentally deranged. Failure of vision probably associated with the disease rather than with the drug.
								Absconded after 5th dose somewhat improved.
								Absconded after 1st dose.
At 10th dose	Appears and feels well. No headaches. Glands very small. Pulse 84. Reflexes and vision normal.	116	—	—	0.040	25	—	
4 weeks after 10th dose	Still quite well. Glands very small. Pulse 84. Reflexes and vision normal.	116	—	—	0.030	16	—	

TABLE II (Continued).—Showing the results of treating patients (with pathological cerebrospinal fluids),

Case	Condition immediately before treatment							Treatment. Gm. neocryl per kgm. body weight × no. of doses. Interval between doses
	Clinical	Body weight in lb.	Blood tryps.	Gland- juice tryps.	Protein %	Cells	Tryps.	
107	1 year. Headaches and somnolence. Deeply lethargic with expressionless face. Unable to walk. Small glands in neck. Pulse 96.	156	—	—	0.255	1,600	+	0.045 × 10 weekly
108	6 months. Headaches and somnolence. Slight generalized tremor. Walks with difficulty. Glands in neck and axillae. Pulse 90.	90	—	+	0.170	1,400	+	0.045 × 10 weekly
110	1 year. Headaches and poor vision. Generalized adenitis. Pulse 104. Fundus normal.	135	—	+	0.120	230	+	0.045 × 10 weekly (Absent 2 weeks between 3rd and 4th doses, 7th and 8th doses, and 9th and 10th doses)
113	6 months. Headaches and somnolence. 5 months. Amenorrhoea. Glands in neck and axillae. Spleen 2" palpable. Pulse 126.	100	—	+	0.270	400	+	0.045 × 10 weekly (Absent 2 weeks between 2nd and 3rd doses)
114	3 years. Headaches and somnolence. Deeply lethargic, with typical facies, sordes and drooling. Small gland in left axillae too small to puncture; none palpable in neck. Pulse 126.	133	—		0.320	1,500	+	0.045 × 10 weekly (Absent 2 weeks between 1st and 2nd doses, and between 2nd and 3rd doses)

uids), suffering from Gambian sleeping sickness, with neocryl

Condition after treatment								Remarks
Time of examination	Clinical	Body weight in lb.	Blood tryps.	Gland-juice tryps.	C.S.F.			
					Protein %	Cells	Tryps.	
At 10th dose	Appears and feels much better. Lethargy much less, and now walks well. No headaches or somnolence. Glands too small to puncture. Pulse 66. Reflexes and vision normal.	150	—		0.100	40	—	
At 10th dose	Appears and feels much better. No headaches or somnolence. Tremor less. Walks well. Pulse 68. Reflexes and vision normal.	98	—	—	0.075	30	—	
At 10th dose	Appears and feels well. No headaches. Glands very small. Pulse 84. Says vision is improved. Reflexes normal.	146	—	—	0.070	22	—	
At 10th dose	Appears and feels well. No headaches or somnolence. Menstruation re-established. Spleen not palpable. Pulse 72. Reflexes and vision normal.	114	—	—	0.150	50	—	
At 10th dose	Appears and feels much improved. Still mentally slow, but better than originally. Pulse 120. Reflexes and vision normal.	161	—		0.090	100	—	

TABLE II (Continued).—Showing the results of treating patients (with pathological cerebrospinal

Case	Condition immediately before treatment							Treatment. Gm. neocryl per km. body weight × no. of doses. Interval between doses
	Clinical	Body weight in lb.	Blood trysps.	Gland- juice trysps.	C.S.F.			
					Protein %	Cells	Tryps.	
115	9 months. Headaches. 3 months. Somnolence. Gen- eralized adenitis. Pulse 84.	119	—	+	0.050	23	—	0.045 × 10 weekly
117	4 months. Loss in weight, occa- sional fevers. Headaches and somnolence. Marked lethargy. Generalized adenitis. Pulse 84.	116	—	+	0.180	500	+	0.045 × 10 weekly (Absent 2 weeks between 4th and 5th doses)
120	1 year. Glands in neck. 4 months. Headaches and somno- lence. Glands in neck and axillae. Pulse 108.	118	—	+	0.060	30	+	0.045 × 5 weekly
122	1 year. Abdominal pain and head- aches. 2 months. Somnolence. Glands in neck and axillae. Pulse 126.	102	—	+	0.100	350	+	0.045 × 10 weekly (Absent 2 weeks between 2nd and 3rd doses)
123	3 years. Glands in neck. 4 months. Somnolence alternat- ing with periods of mania. Deeply somnolent, with mania- cal outburst requiring severe physical restraint. Generalized adenitis. Pulse 120.	75	—	+	0.100	325	+	0.045 × 9 weekly

fluids), suffering from Gambian sleeping sickness, with neocryl

Condition after treatment								Remarks
Time of examination	Clinical	Body weight in lb.	Blood tryps.	Gland-juice tryps.	C.S.F.			
					Protein %	Cells	Tryps.	
At 10th dose	Appears and feels well. No headaches or somnolence. Glands very small. Pulse 78. Reflexes and vision normal.	117	—		0.040	5	—	
At 10th dose	Appears and feels well. Lethargy gone. No headaches or somnolence. Glands small. Pulse 66. Reflexes and vision normal.	118	—	—	0.045	52	+	Clinically improved and protein and cell content of C. S. F. improved, but tryps. still present. Recommended further treatment, but absconded.
								Absconded after 5th dose somewhat improved.
At 10th dose	Appears and feels well. No headaches or somnolence. Glands very small. Pulse 120. Reflexes and vision normal.	112	—	—	0.060	150	—	
2 weeks after 10th dose	Still quite well.		—	—	0.060	102	—	
1 week after 7th dose	Physically and mentally improved, but still talks a lot. Glands smaller. Pulse 120. Reflexes and vision normal.	70	—	—	0.040	35	—	Absconded after 9th dose very much improved.

TABLE II (Continued).—Showing the results of treating patients (with pathological cerebrospinal

Case	Condition immediately before treatment							Treatment. Gm. neocryl per kgm. body weight × no. of doses. Interval between doses
	Clinical	Body weight in lb.	Blood tryps.	Gland- juice tryps.	C.S.F.			
					Protein %	Cells	Tryps.	
124	1 year. Pains in chest and headaches, with slight somnolence. Lethargic. Generalized adenitis. Spleen 2" palpable. Pulse 96.	104	—	+	0.045	45	+	0.045 × 11 weekly (Absent 2 weeks between 2nd and 3rd doses)
125	1 year. Headaches and somnolence. Lethargy marked and hardly able to walk. Generalized adenitis. Pulse 96.	92	—	+	0.080	520	+	0.045 × 10 weekly (Absent 2 weeks between 6th and 7th doses)
127	4 months. Generalized pains, headaches and occasional fever. 3 months. Somnolence. Moderate-sized glands in neck and axillae. Pulse 102.	103	—	+	0.050	100	+	0.045 × 10 weekly (Absent 2 weeks between 3rd and 4th doses)

fluids), suffering from Gambian sleeping sickness, with neocryl

Condition after treatment								Remarks
Time of examination	Clinical	Body weight in lb.	Blood tryps.	Gland-juice tryps.	C.S.F.			
					Protein %	Cells	Tryps.	
At 10th dose	Appears and feels quite well. No pain, headaches or somnolence. Glands too small to puncture. Spleen only just palpable. Pulse 96. Reflexes and vision normal.	124	—		0.030	0	—	
At 10th dose	Appears well.							Completed treatment, but went off and did not return before lumbar puncture was done.
At 10th dose	Appears well. No fever, headaches or somnolence. Glands very much smaller. Pulse 72. Reflexes and vision normal.	112	—	—	0.060	22	—	

CASES WITH ABNORMAL SPINAL FLUIDS

The observations on these cases are summarized in Table II (pp. 492-525), which reveals the following facts.

These cases totalled 78, of which 46 received a course of 10 or more injections. Of the 46 cases completing a course, 45 showed definite clinical improvement at the end of treatment, and this was supported in 35, by finding an improved condition of the spinal fluid.

The patient failing to improve clinically (Case 98) still had headaches at the end of treatment and was beginning to get occasional attacks of somnolence. Furthermore, during the course of treatment her mentality had deteriorated, a generalized tremor had developed and her vision had become poor. The visual failure appeared to be associated with corneal changes which are discussed later, and in view of its late onset was possibly due to the progress of the disease rather than to a toxic action of the drug, but it was not possible to determine this. It is interesting that the protein content and cell count of her cerebrospinal fluid improved from 0.300 per cent. and 400 per c.mm. before treatment, to 0.070 per cent. and 94 per c.mm. respectively after treatment, and very careful examinations of the centrifuged deposit from the spinal fluid, of the gland-juice and of the blood failed to reveal any trypanosomes.

Of the ten patients in which the improvement at the end of treatment was only demonstrated clinically, three (Cases 31, 44 and 125) absconded before lumbar puncture could be carried out, and so nothing is known of the response of their cerebrospinal fluids. Of the remaining seven, the cell count of the spinal fluid had increased in three (Cases 4, 6 and 77), and in one other (Case 48) the protein in addition, although the trypanosomes had disappeared. Van Slype (1935), Barlovatz (1935), Sicé (1937) and others have recorded increases in protein and cells following treatment, which they regarded as the results of a meningeal reaction caused by the drugs used, since the values fell later without further treatment. It is interesting to note that of the four cases above, three (Cases 6, 77 and 48) had had repeated lumbar punctures during the course of treatment, and it is possible that this had something to do with the changes in the protein content and cell count of the spinal fluids. It seemed in certain instances as if the protein content and cell count rose during the earlier stages of treatment and fell towards normal later, but the relative parts played by the disease, the drug or the trauma in this reaction were not determined. The three remaining patients who improved only clinically (Cases 24, 38 and 117) had trypanosomes in their cerebrospinal fluids at the time of the ninth or tenth dose, although the protein content and cell count of the fluids had diminished. One of these (Case 117) was clinically so well at the end of the course that he absconded, in spite of being strongly advised to attend for further treatment. In another (Case 38) trypanosomes were found in the spinal fluid nine weeks after commencing treatment. The dose was immediately doubled

to 0.09 gm. per kgm. body weight, and five such doses were given at weekly intervals without any toxic effect. Nine weeks after this further course the protein content and cell count of the spinal fluid were found to be again increased, but no trypanosomes were seen, and the patient, apart from some loss in weight, was otherwise clinically well. The other patient (Case 24) also had trypanosomes in the spinal fluid at the time of the ninth dose, although the cell count of the fluid had improved. He was given an increased dosage of 0.075 gm. per kgm. body weight, but this produced vomiting and abdominal pain. The following dose was therefore reduced to 0.06 gm. per kgm. body weight, and this was repeated weekly without untoward effect for three weeks. The protein content and cell count of the cerebrospinal fluid were practically uninfluenced at the end of this second course of treatment, but the trypanosomes had apparently disappeared, and he was still clinically quite well when seen seven weeks later.

Toxic Effects. In addition to the patient referred to above (Case 24), who had vomiting and abdominal pain after a large dose of 0.075 gm. per kgm. body weight, five others had symptoms which might possibly have been due to a toxic action of the drug, but in only three was it probable that the drug was the direct cause of the symptoms.

Of these five patients, one (Case 33), after the first dose of 0.045 gm. per kgm. body weight, had some abdominal pain and vomiting, which passed off after a few days and did not recur, although treatment was not interrupted until seven doses had been given, when the patient absconded apparently well. Another patient (Case 20) complained of transient dizziness five weeks after commencing treatment, but a week later stated that he was quite well, and it was uncertain that the drug had anything to do with the attack.

The remaining three patients (Cases 74, 84 and 98) suffered visual disturbances. Case 74 had only slight diminution of vision after the first dose of 0.045 gm. per kgm. body weight, from which she recovered within a week, although treatment was not interrupted until the fourth dose, when she absconded, as far as was known clinically well and with normal vision. Case 84 absented himself for eight weeks after the fourth dose, owing to failure of vision. Since at his return the vision was completely restored and the fundi appeared normal, treatment was reinstituted. This patient absconded after eight doses, although he still had slight pains in the neck and the protein content and cell count of his cerebrospinal fluid were still abnormal, but during the time he attended for treatment there was no further visual trouble. Case 98, whose condition gradually deteriorated, and to whom reference has already been made, was absent for three weeks after the ninth dose, and when she returned was found to be suffering from serious impairment of vision. The corneae were diffusely hazy, but the fundi, although seen only indistinctly, as far as could be ascertained presented no gross changes. In view of the late impairment of vision, the corneal condition and the general evidence of failure of treatment, it is possible that the loss of sight was due to the disease invading the corneae, and probably

not to any toxic action of the drug. Keratitis in untreated cases of sleeping sickness has been mentioned by Daniels (1912) and Hissette (1932), and Morax (1907) and Yorke (1911) have demonstrated invasion of the cornea by trypanosomes in experimental trypanosomiasis of animals.

Relapses. In addition to the four patients (Cases 24, 38, 98 and 117) already referred to, who responded imperfectly to treatment, four others (Cases 10, 11, 13 and 60) relapsed at various periods after a course of neocryl.

Case 10, who had received doses of 0.055 gm. per kgm. body weight weekly for ten weeks, was examined nine weeks after the end of the treatment, but, although he was clinically well and no trypanosomes were to be found in the blood, gland-juice or cerebrospinal fluid, the protein content and cell count of the spinal fluid were 0.100 per cent. and 250 per c.mm., showing a definite regression from the values obtained immediately treatment was finished, when they were 0.035 per cent. and 22 per c.mm. respectively. A fortnight after this examination, it was reported that seven days previously—namely, ten weeks after finishing treatment—the patient had suddenly become ill, complaining of severe headache, and had died within three days. The cause of death was uncertain, but the condition of the spinal fluid shortly before, and the history, make it necessary to include the case as a relapse.

Case 11, who had received the same dosage as the previous patient, was clinically well and the spinal fluid was normal, except for a slightly raised cell count of 15 per c.mm., immediately after finishing treatment. Seven weeks later she returned complaining of headaches of six days' duration and vomiting of three days' duration. She was lethargic, had lost 8 lb. in weight, the pulse rate was 104, the spleen was 2 inches palpable, but the glands were extremely small. The spinal fluid contained trypanosomes, and the protein content and cell count were very much increased to 0.24 per cent. and 600 per c.mm. respectively. She was given a further course of 0.055 gm. per kgm. body weight, and was clinically so much improved after three doses that she absconded and was not seen again.

Case 13 was an advanced case with dementia and was treated with 10 weekly doses of 0.065 gm. per kgm. body weight. At the end of treatment she was clinically well, mentally improved and leading a normal life. The protein content and cell count of the spinal fluid were, however, still raised, being 0.070 per cent. and 40 per c.mm. respectively, but no parasites were found. Fifteen weeks later she complained of occasional headaches and slight somnolence. There was a generalized tremor, but mentally she still seemed normal. Trypanosomes were again not found, but the protein content and cell count of the spinal fluid were further raised to 0.090 per cent. and 130 per c.mm. respectively.

Case 60 received 11 weekly injections of 0.045 gm. per kgm. body weight, and at the end of treatment was apparently normal, except for a raised cell count of 66 per c.mm. in the cerebrospinal fluid. Ten weeks later she reported that

during the previous fortnight she had had occasional headaches, and on examination trypanosomes were found in the spinal fluid, and the protein content and cell count were considerably increased, being 0.065 per cent. and 570 per c.mm. respectively.

SUMMARY

Neocryl produced a rapid and definite clinical improvement in practically every case treated, although some of the patients were in a very advanced stage of disease. Almost without exception patients reported an immediate improvement after only one or two injections, and the headaches and somnolence soon disappeared. The general appearance and facies became normal, the cerebration brighter, and patients who were helpless and carried in for their first injection were able to walk within a week or two. Usually there was a gain in weight, which in some instances amounted to two stones by the end of treatment. A considerable number of patients felt so well after a few doses that they absconded.

Of the early cases with normal spinal fluids, 30 finished a course of treatment, all were clinically improved, two experienced visual disturbances from which complete recovery was made, and one relapsed sixteen weeks after finishing treatment.

Of the latter cases with pathological spinal fluids, 46 finished a course of treatment, and of these 35 were clinically improved and their spinal fluids also underwent a change towards normality. One case failed to improve clinically, and in three others trypanosomes failed to disappear from the spinal fluid, although the patient's general condition was improved. In four cases the patients improved clinically and the parasites disappeared from the spinal fluid, but either the protein content or the cell count was not improved. In three cases which improved clinically the spinal fluid was not examined at the end of treatment. Three patients receiving routine doses suffered from toxic effects which were attributed to the drug, one having an attack of vomiting and abdominal pain, and two experiencing transient disturbances of vision, which in one case was very slight. Another patient had gastro-intestinal symptoms after a large dose, and another had a short attack of dizziness which could not definitely be associated with the drug, while yet another suffered loss of vision which was probably due to the disease rather than the drug. Four cases relapsed during the period of observation, which varied from patient to patient, but in some instances was over four months.

These results resemble those which one might expect to have obtained if tryparsamide had been used, and probably the two drugs are very similar in their actions. It is interesting to note, however, that Hawking, Hennelly and Quastel (1937) have recently stated that, although approximately the same amounts of arsenic are found in the cerebrospinal fluid after equal doses of neocryl or tryparsamide, the trypanocidal activities of such fluids are markedly

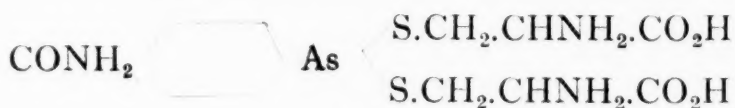
different. After a dose of tryparsamide the fluid exhibits an appreciable trypanocidal titre, but after a dose of neocryl the trypanocidal titre is only slight or negligible. They believe that the active compound found in the cerebrospinal fluid after a dose of tryparsamide is derived from reduction of the drug occurring in the brain, whereas in the case of neocryl, although this drug reaches the brain, it fails to be reduced or converted into a trypanocidally active condition. This somewhat puzzling anomaly is interesting, since neocryl appeared, at least in the earlier tests, to be more active against all stages of somatic syphilis than did tryparsamide. Together these observations suggest that either the mechanism of action against spirochaetes differs from that against trypanosomes, or that the conditions in the brain are peculiarly selective and different from those in the general tissues or blood.

It is, however, impossible to draw any accurate comparison between the relative values of the two drugs in sleeping sickness until the later fate of cases treated with neocryl is known. Arrangements have been made with the various medical officers in the Gambia to continue the observation of patients of the present series where possible.

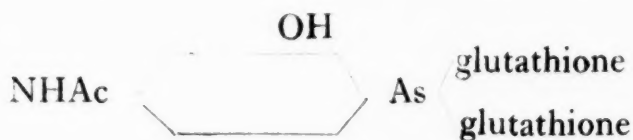
K.324 AND K.352

These two aromatic thioarsinites were prepared by Dr. King and his colleagues, and are of the following constitution :

K.324, di(β -carboxy- β -aminoethyl) benzamide-p-thioarsinite



and K.352, di-glutathionyl-4-acetamino-2-hydroxyphenyl thioarsinite



Strangeways (1935) reported that they were effective in curing *T. rhodesiense* and *T. gambiense* infections in mice in doses which were only a fraction of the maximum tolerated. They were also active against experimental infections in rabbits in relatively small doses, three to six doses of 0.01 gm. K.324 per kgm. body weight, or four to eight doses of 0.01 gm. K.352 per kgm. body weight, at three-day intervals, producing permanent cures in rabbits infected with her strain of *T. rhodesiense*, and she considered that they had peculiar advantages which merited further exploration because of the small amount of arsenic contained in them.

As nothing was known of the dosage applicable to man, it was necessary to proceed with caution in the present experiment, and to modify the dose and intervals according to the reaction of the patients. Soluble sodium salts of the drugs were used and were administered intravenously, usually in 5 per cent. solution, in freshly distilled water. It was necessary to heat K.324 in order to effect solution, the mixture of drug and water being brought just to the boil, during which process the drug dissolved with effervescence; it was then rapidly cooled, overheating being always carefully avoided. K.352 was dissolved in cool sterile distilled water.

K.324

The observations made on the action of this drug are summarized in Table III (pp. 532-537), from which the following facts emerge.

Solutions of K.324 frequently produced thrombosis at the site of injection, although considerable care was taken over the injection. Doses of more than 2.5 mgm. per kgm. body weight usually produced toxic reactions, vomiting, abdominal pain and diarrhoea being common with the smaller doses, while with larger doses there was, in addition, albuminuria with casts, and in one case severe jaundice. Eight cases were treated with doses ranging from 2.5 mgm. per kgm. upwards, but in no instance was there any considerable clinical improvement. Diminution in the protein content or cell count of the spinal fluid was slight and of a temporary nature, the condition rapidly regressing while parasites either persisted or reappeared in the fluid within two or three weeks after the cessation of treatment.

K.352

The results obtained with K.352 resembled those with K.324, and the observations made on eight cases treated with this drug are summarized in Table IV (pp. 538-541). From this table it can be seen that, even with doses of only 2.5 mgm. per kgm. body weight, vomiting and diarrhoea occasionally occurred, while with larger doses there was, in addition, a toxic albuminuria. The drug in maximum tolerated doses failed to produce more than slight clinical improvement, and the effect on the cerebrospinal fluid was negligible.

One patient (Case 4) after a single dose of 2.5 mgm. per kgm. body weight vomited, developed tachypnoea and hyperthermia, contraction of the right sternomastoid muscle, and marked tremor of left arm and leg, and died shortly afterwards. Although such a dose gave rise in other cases to vomiting and diarrhoea, these reactions were relatively slight, and it is possible that this unfortunate result was another example of a Jarisch-Herxheimer reaction.

SUMMARY

The two drugs, K.324 and K.352, were found to be toxic for man in relatively small doses, and all doses up to the maximum tolerated were ineffective in the therapy of human cerebral trypanosomiasis.

TABLE III.—Showing the results of treating patients

Case	Condition immediately before treatment							Treatment. Mgm. K.324 per kgm. body weight × no. of doses. Interval between doses
	Clinical	Body weight in lb.	Blood tryps.	Gland- juice tryps.	C.S.F.			
					Protein %	Cells	Tryps.	
1	3 months. Malaise and headaches. No somnolence. Moderate- sized glands in neck and axillae. Pulse 120.	153	—	+	0.030	100	+	2.5 × 7, at 3- to 4- day intervals.
2	1 month. Generalized pains, loss in weight. Headaches and som- nolence. Moderate-sized glands in neck and axillae. Pulse 120.	139	—	+	0.045	330	+	5.0 × 5 2.5 × 2 at 3- to 5-day intervals (7 days between 1st and 2nd dose)
3	8 months. Generalized pains, loss in weight, headaches and irregu- lar fever. 5 months. Somnolence. Moder- ate-sized glands in neck. Pulse 126.	100	—	+	0.090	105	+	7.5 × 9, at 3-day intervals (5 and 6 days between 1st and 2nd and 2nd and 3rd doses respectively)
4	4 months. Headaches and occa- sional fever. 3 months. Somnolence. Lethar- gic. Generalized adenitis. Pulse 120.	59	—	+	0.040	84	+	15.0 × 1 7.5 × 1 5.0 × 4 2.5 × 1, intervals between doses were 21, 3, 7, 4 3 and 7 days respectively.

suffering from Gambian sleeping sickness with K.324

Condition after treatment								Remarks
Time of examination	Clinical	Body weight in lb.	Blood tryps.	Gland-juice tryps.	C.S.F.			
					Protein %	Cells	Tryps.	
3 days after 7th dose	Thrombosis at site of injection followed 2nd dose. Headaches less. Glands smaller. Pulse 120. Thrombosis improving.	148	—	—	0.040	80	—	Clinical improvement slight, and 3 weeks after last dose protein and cell content of C. S. F. were worse, although no trypanosomes were found.
10 days after 7th dose		146			0.050	50	—	
21 days after 7th dose		149	—	—	0.060	140	—	
2 weeks after 7th dose	Vomiting followed each injection. Vomited after 6th injection. Still has headaches, but somnolence somewhat less. Glands smaller. Pulse 102.	142	—	—	0.060	900	+	Clinical improvement, negligible, and 2 weeks after last dose the condition of C. S. F. was worse and tryps. were present.
3 days after 9th dose	Occasional vomiting followed the injections. Lethargic. Glands smaller. Pulse 120. Clinically unchanged. Clinical improvement negligible. Pulse 96.	100	—	—	0.090	84	—	No clinical improvement, and trypanosomes again present in C.S.F. 21 days after end of course.
11 days after 9th dose		103			0.100	104	—	
21 days after 9th dose		100	—	—	0.090	400	+	
3 weeks after 7th dose	Severe vomiting and diarrhoea followed earlier injections, and occasional vomiting followed the smaller doses. Appears better and lethargy less. Headaches and somnolence somewhat less. Glands smaller. Pulse 90.	60	—	—	0.040	170	—	Some clinical improvement, but C. S. F. cell count increased and protein content uninfluenced, although doses were maximal.

TABLE III (Continued).—Showing the results of treating patients suffering

Case	Condition immediately before treatment							Treatment. Mgm. K.324 per kgm. body weight × no. of doses. Interval between doses
	Clinical	Body weight in lb.	Blood tryps.	Gland- juice tryps.	C.S.F.			
					Protein %	Cells	Tryps.	
5	3 months. Generalized pains. Loss in weight, headaches and somnolence. Lethargic appearance. Moderate to large glands in neck and axillae. Pulse 90.	113	—	+	0.140	800	+	10.0 × 8, at 3-day intervals.
6	4 months. Headaches. Moderate-sized soft glands in neck and axillae. Pulse 114.	116	—	+	0.045	260	—	15 × 1 10 × 1 7.5 × 5 intervals between doses were 6, 5, 7, 3, 4 and 3 days respectively.
7	4 months. Generalized pains and headache. 1 month. Somnolence. Moderate-sized glands in neck and axillae. Pulse 138.	100	—	+	0.080	450	+	15.0 × 2 10.0 × 1 5.0 × 4 intervals between doses were 5, 6, 6, 3, 3 and 3 days respectively.

from Gambian sleeping sickness with K.324

Condition after treatment								Remarks
Time of examination	Clinical	Body weight in lb.	Blood tryps.	Gland-juice tryps.	C.S.F.			
					Protein %	Cells	Tryps.	
3 days after 8th dose	Thrombosis at sites of injections, and occasional vomiting followed injections. Still lethargic, but headache less. Glands still present. Pulse 78.	112	—	—	0.090	300	—	Clinical improvement negligible, and trypanosomes found in C.S.F. 11 days after end of course.
11 days after 8th dose	Still lethargic and clinical condition much as originally.	116	—	—	0.160	750	+	
At 7th dose	Persistent vomiting, severe abdominal pain and heavy albuminuria followed injection. Vomited after injection. Headaches less and feeling somewhat improved, but progressively losing weight.	109						Some clinical improvement, but absconded after 7th dose before examination of blood, gland-juice and C.S.F. could be carried out.
At 7th dose	Vomiting and abdominal pain followed each injection; and pain at site of 2nd injection followed this dose. Vomiting followed injection. Painful thrombosis at site of injection. Feels somewhat improved and headaches are less. Glands a little smaller. Pulse 120.	99	—	—	0.060	275	—	Slight clinical improvement, but even at 7th dose trypanosomes still found in C.S.F. Also protein and cell content of fluid relatively uninfluenced.
1 week after 7th dose	Still feeling better and headaches less.	99	—	—	0.090	285	+	

TABLE III (Continued).—Showing the results of treating patients suffering

Case	Condition immediately before treatment							Treatment. Mgm. K.324 per kgm. body weight × no. of doses. Interval between doses
	Clinical	Body weight in lb.	Blood tryps.	Gland- juice tryps.	C.S.F.			
					Protein %	Cells	Tryps.	
8	1 year. Glands in neck and loss in weight.	100	—	+	0.050	80	+	10.0 × 1
	6 months. Headache.							17.5 × 1
	4 months. Somnolence. Generalized adenitis. Spleen 2" palpable. Pulse 150.							22.0 × 1
								25.0 × 1
								35.0 × 1 at 5-day intervals

from Gambian sleeping sickness with K.324

Condition after treatment								Remarks
Time of examination	Clinical	Body weight in lb.	Blood tryps.	Gland-juice tryps.	C.S.F.			
					Protein %	Cells	Tryps.	
18 hours after 1st dose	Pain and induration at site of injection.	95	—	—	0.050	100	+	Despite short intervals between doses it is possible resistance developed during treatment, but 10 mgm. was ineffective.
Day following 2nd dose	Albuminuria with casts.							
Day following 3rd dose	Albuminuria less.							
Day following 4th dose	Urine normal.							
Day following 5th dose	Abdominal pain, followed by slight icterus.	88	—	—	0.050	24	—	
3 weeks after 5th dose	Jaundice clearing. Headaches and somnolence less. Glands smaller. Pulse 100.		—	—	0.050	34	—	
4 weeks after 5th dose	No headache and feels better. Jaundice practically cleared.	93	—	—	0.090	145	+	
8 weeks after 5th dose	Headaches returned. Marked loss in weight.	74						

TABLE IV.—Showing the results of treating patients

Case	Condition immediately before treatment							Treatment. Mgm. K.352 per kgm. body weight × no. of doses. Interval between doses
	Clinical	Body weight in lb.	Blood tryps.	Gland- juice tryps.	C.S.F.			
					Protein %	Cells	Tryps.	
1	9 months. Generalized pains, loss in weight, headaches and somnolence.	94	—	+	0.100	110	+	2.5 × 7, intervals between doses were 5, 3, 5, 8, 4 and 3 days respectively
2	6 months. Headaches. 4 months. Somnolent, lethargic and mentally impaired. Small to moderate-sized glands in neck and axillae. Pulse 116.	161	—	+	0.040	200	—	2.5 × 7, at 3- to 4-day intervals
3	3 months. Headaches and somnolence. Lethargic. Difficulty in walking. Marked generalized tremor. Moderate-sized glands in neck. Pulse 120.	154	—	+	0.100	250	++	2.5 × 10, at 3- to 4-day intervals
4	2 months. Headaches, occasional fever and increasing somnolence. Lethargic. Small glands in neck. Pulse 84.	80	—	+	0.100	170	+	2.5 × 1

suffering from Gambian sleeping sickness with K.352

Condition after treatment								Remarks
Time of examination	Clinical	Body weight in lb.	Blood tryps.	Gland-juice tryps.	C.S.F.			
					Protein %	Cells	Tryps.	
At 7th dose 1 week after 7th dose	Some diarrhoea followed middle doses. Still getting headaches. Headaches and somnolence less. Glands smaller. Pulse 120.	93	—	—	0.050	93		Slight clinical improvement, but C. S. F. still abnormal.
1 week after 7th dose	No clinical improvement. Still getting headaches, and mental condition unchanged. Pulse 102. Urine —trace of albumen.	148	—	—	0.045	198	—	Clinical improvement negligible and progression of disease definite, as shown by C.S.F. examination.
3 weeks after 7th dose	Says headaches are less and appears somewhat better, but still very dull. Pulse 116.	147	—	—	0.100	700	+	
11 days after 10th dose	Feels and looks better. Walking improved. Headaches and somnolence less. Still has generalized tremor. Glands smaller. Pulse 96. Urine n.a.c.	150	—	—	0.140	210	+	Clinically improved, but C.S.F. uninfluenced.
	Vomited during injection. During the next few days there was marked tachypnoea, hyperthermia, inability to stand, contraction of rt. sternomastoid and marked tremor of left arm and leg, followed by death on 4th day.							Possibly a Jarisch-Herxheimer reaction

TABLE IV (Continued).—Showing the results of treating patients suffering

Case	Condition immediately before treatment							Treatment. Mgm. K.352 per kgm. body weight × no. of doses. Interval between doses
	Clinical	Body weight in lb.	Blood tryps.	Gland- juice tryps.	C.S.F.			
					Protein %	Cells	Tryps.	
5	5 months. Generalized pains and headaches. Small to moderate-sized glands in neck and axillae. Pulse 104.	149	—	+	0.080	400	+	5.0 × 1 2.5 × 6 at 3- to 4-day intervals (7 days between 1st and 2nd dose)
6	6 months. Headaches and somnolence. Moderate-sized soft glands in neck. Pulse 102.	133	—	+	0.060	62	+	10.0 × 1 and after 6 days 5.0 × 7, at 3-day intervals
7	1 year. Glands in neck and occasional fever. 3 months. Somnolence. Generalized adenitis.	74	—	+	0.080	500	+	10.0 × 1
8	3 months. Headaches. 2 months. Somnolence. Lethargic. Small to moderate-sized glands in neck and axillae. Pulse 76.	116	—	+	0.060	235	+	7.5 × 1 10.0 × 2 at 5- and 4-day intervals

from Gambian sleeping sickness with K.352

Condition after treatment								Remarks
Time of examination	Clinical	Body weight in lb.	Blood tryps.	Gland-juice tryps.	C.S.F.			
					Protein %	Cells	Tryps.	
2 weeks after 7th dose	Vomiting and occasional diarrhoea followed many of the injections. Feels better and headaches are less. Glands smaller. Pulse 96.	148	—	—	0.090	425	—	Some clinical improvement, but protein and cell content of C.S.F. uninfluenced, although trypanosomes could no longer be found.
3 days after 8th dose	1st injection was followed by abdominal pain, diarrhoea and severe albuminuria. Occasional vomiting after some of smaller doses. Headaches less. Glands smaller. Pulse 90. Urine n.a.c.	131	—	—	0.050	128	—	Clinical improvement slight and no marked improvement in protein and cell content of C. S. F., although trypanosomes were not found in fluid.
2 weeks after 8th dose	Again complaining of headaches. Pulse 156.	129	—	—	0.050	51	—	
3 weeks after 8th dose	Occasional headaches.	123	—	—	0.045	123	—	
6 weeks after 8th dose	Headaches less and somnolence less than originally. Pulse 96.	128	—	—	0.045	100	—	
18 hours after dose	Severe albuminuria with casts, which persisted for some days.		—	—	0.070	320	+	
At 3rd injection	Slight albuminuria after 1st dose. Considerable temperature following 3rd dose, with abdominal pain, vomiting, severe diarrhoea and epistaxis. Heavy albuminuria.				0.090	125	+	Toxic effect of 3rd dose very severe and recovery slow. 4 days after 2nd of two maximal doses trypanosomes were still present in C.S.F.

ACKNOWLEDGEMENTS.—My thanks are due to the Gambian Government and the Colonial Office for the hospitality afforded to me in the Gambia ; and I am particularly indebted to the Senior Medical Officer of the Gambia and the members of his department for their generous help in these investigations.

I should also like to thank Messrs. May and Baker, Ltd., and Dr. Ewins for the generous supplies of 'Crylarsan brand of neocryl.'

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